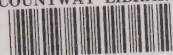


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PROTOZOA AND DISEASE

PART I.



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# PROTOZOA AND DISEASE

BY

J. JACKSON CLARKE

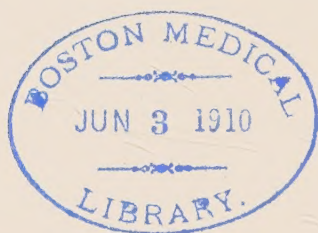
M.B. LOND.

AUTHOR OF 'SURGICAL PATHOLOGY AND PRINCIPLES' ETC.

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TO

SIR WILLIAM HENRY BROADBENT, BARONET,

K.C.V.O., F.R.S., LL.D., M.D.



## P R E F A C E

THIS volume covers the same ground as was covered by three papers that appeared under the heading 'The Protozoa considered in Relation to Disease,' in the *Medical Press and Circular*, August 16, 23, and 30, 1893, and I subsequently discussed the same subject in some lectures delivered at the Medical Graduates' College and Polyclinic in 1901. During the years that have elapsed since 1892, when, in the course of my work as pathologist at one of the large general hospitals of London, I was first brought into touch with the pathological relations of the protozoa, many important contributions to the subject have appeared in different parts of the world. In the present work I have fully availed myself of these additions to literature, and it has been my aim, by giving references, to enable those who may wish to do so to consult the original documents.

J. JACKSON CLARKE.

18, PORTLAND PLACE,  
LONDON, W.

*May 1, 1903.*



## INTRODUCTION

THE idea underlying the term 'contagion' in pathology is the supposition that some morbid material is transferred from a diseased to a previously healthy individual, or from an infected to a previously healthy part of the body of the same individual. Long before it was proved that diseases such as tuberculosis, typhoid fever, diphtheria, anthrax, etc., were due to the transference of living vegetable organisms, the hypothesis of a *contagium vivum* was in existence, and the term 'zymotic,' or fermentative, was applied to the best-known of the infective fevers. At the beginning of the past century the doctrine of irritation and of inflammation was dominant in pathology.

The differences presented by inflammatory disorders were explained by the greater or less intensity of the irritating agent, or of the more or less active reaction of the economy; as to the quality of the agent, no question was raised. A common catarrh of the intestine only differed from typhoid fever or cholera by the varying degree of sympathetic irritation as evidenced by the symptoms; there was no thought of any difference in the underlying causes. In France, Laënnec and Bretonneau first established the conception of specificity in place of 'physiologism' in pathology and mock rationalism in

therapeutics. With Trousseau\* the quality of the underlying cause assumed the foremost place in pathology. Diphtheria, typhoid, the eruptive fevers, and many other diseases, present special characters, which differentiate them from one another and allow of the classification of disease in as absolute a manner as that of botanical or zoological species. Thus arose the name of specific diseases. Trousseau† predicted in explicit terms the advent of bacteriology :

‘I must remind you of the new theory of ferments of Pasteur. You know that this savant has come to deny the existence of ferments; minutely careful experimentation has led him to pronounce that fermentation is caused by spores, and that for each kind of fermentation there is a special spore, recognisable by certain characters, and endowed with the property of causing in suitable media a particular kind of fermentation. In this way there would be different spores for the alcoholic, the lactic, and the butyric fermentations, and so forth. Now, may there not exist also *morbid spores*, spores that only await certain definite conditions to reveal their existence—to develop, multiply, and to give birth to, say, a morbid fermentation? Has it not been said that pus causes pus? Perhaps there is a *special sporule that accounts for the purulent infection; is there also a dysenteric sporule, a sporule of cholera, etc.?* The facts of contagion would thus be accounted for if one could find the presence of these morbid spores; *but to attain that discovery it will be necessary to follow the path that Pasteur has made, and*

\* Trousseau died in 1867.

† G. Dieulafoy, ‘Manuel de Pathologie Interne,’ 13th edition, vol. iv., p. 799.

*to proceed with the same skill and the same experimental patience.'*

The latent or incubation period of the specific fevers was compared by Trousseau to the period that intervenes between a sowing of seed in suitable soil and the appearance of the young leaves above ground; nor was he at all inclined to allow the seed (the microbe) too preponderant a part, but he always insisted upon the importance of the soil—the organization of the patient—which gives to every instance of disease an individual character by reason of the degree in which inherited or acquired powers of resistance to the multiplication of germs are present in the tissues. Now, it is just in regard to the common exanthematous fevers, measles, scarlet fever, chicken-pox, small-pox, etc., that bacteriological methods, even in the ablest hands, have failed to yield any satisfactory results. It would thus appear that different methods of investigation are required for the elucidation of the nature of these diseases, which are of such serious import to the human race, and which are so closely interwoven with human existence that under present conditions they appear to be inevitable. In the case of one of the most typical of them, small-pox, the only safeguard against the disease is to give it in the modified form of vaccinia. In regard to these fevers, as well as other diseases, a considerable amount of work has been done pointing to their being, not of bacterial, but of protozoan origin. One disease, malaria, is known to be caused by protozoa, and were it only to be able to appreciate the place that the malaria parasites occupy in nature, it would be necessary to study the protozoa systematically, with especial stress upon those that are known to be pathogenetic.

What a salutary effect on medical thought and practice the solution of the etiology of malaria has had may be realized by recalling the speculations that were almost accorded the position of proven facts but a few years ago, when at examinations students were expected to explain the action of quinine in lowering the fever of malaria as consisting in a property of binding the oxygen to the red corpuscles, and thus preventing its being used for the production of pyrogenetic substances in the blood. Now we know that the drug kills the young brood of parasites, and that the periodicity of the febrile attacks is due, not to the periodicity of physiological processes in the patients, but simply to the time occupied in the formation of successive broods of the protozoa that cause the disease.

The periodicity of ague is no more remarkable than the striking periods of the infective fevers referred to above. The incubation period, with its vague disturbances of health, the period of invasion, terminated by that of eruption, all point to an infection by parasites of a more complicated life-history than any bacteria. In order to follow the interesting investigations into the nature of certain structures that occur in and among the cells of the tissues, and in the blood in small-pox and other fevers, it is necessary to study the outlines of what is known of the parasitic protozoa. The sequelæ of these exanthems are more fatal immediately or remotely than the diseases themselves. These sequelæ are due to common pyrogenetic bacteria, and the breaches which serve for their inroads are made by the original infecting organisms. It is far more likely that the first attack is made by protozoa than by bacteria.

The aggressive, highly-specialized parasitism of some of the protozoa gives them a totally different import from the more passive bacteria and fungi. The pyogenetic bacteria, that are of prime importance in wounds where the protecting epithelia are broken, are often harmless on unbroken surfaces.

Clinical experience has enabled us to include syphilis among the exanthematous fevers; and in this disease, too, some workers have found appearances that they have interpreted as protozoa. At present much of this work on the fevers is vague and of a character to cause irritation to skilled biologists. It is, however, to be remembered that medicine is still a 'mother of sciences,' and that, roughly, twenty years were required for bringing our knowledge of malaria to its present degree of completeness, and that the process involved the increase, not only of pathological, but also of biological knowledge—a two-sided inertia, which fully accounts for the length of time that, in the absence of a touchstone, such as Koch's postulates, has been required for our enlightenment.

Syphilis, like the other exanthems, has its periods, which, though protracted, are as definite as those of the other kindred fevers, and, like them, point to a complicated life-history in the parasites that cause the disease.

Clinical study has enabled us to class syphilis with the exanthems; as yet little attention has been given to the possibility that the other exanthems may, like syphilis, be continued long after the febrile stages are passed, and may be signalized by lesions twenty years or more after the original disease has appeared to terminate. Some

of the later lesions of syphilis approach the malignant growths in character more closely than anything else, and it is possible that these are due to the survival in the tissues of some of the parasites that cause the common fevers. Such speculations are only allowable when they are used to direct lines of inquiry, the object of which is to establish facts by observation and experiment. Progress in any science of observation, like pathology, must be *from the known to the unknown*. The present volume has been written to bring together the salient points of what has been already definitely ascertained with regard to the protozoa as parasites and pathogenetic organisms. To biologists much of this small book must appear rough and elementary, but it may serve to give them an insight into the point of view of those who are concerned with pathology. To medical men it is hoped that it may be of some use as a basis for considering recent and forthcoming work on the protozoa in disease. It is proposed in a second part to collect the work that has been done with regard to the part alleged, but not as yet fully proved, to be played by protozoa in diseases some of which are mentioned above.

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# PROTOZOA AND DISEASE

## CHAPTER I

### UNICELLULAR ORGANISMS AND THE CELL

ORGANISMS which throughout their life consist of a single cell comprise individuals, some of which are of distinctly vegetable, others of distinctly animal characters. The former are grouped together as the *protophyta*, the latter as the *protozoa*. Some forms cannot yet with certainty be classed, because their characters are not sufficiently pronounced to mark them out either as plants or animals. The *protophyta* comprise the unicellular algæ and the unicellular fungi, the latter including the bacteria and the blastomycetes, which, on account of their proved importance in pathology, have become familiar to all. Not so the *protozoa*. 'What are the *protozoa*?' is a question that is asked not infrequently, even by medical men. They are the minute animal organisms, some of which were discovered by means of simple lenses of his own making by Leeuwenhoek \* (1632-1723) 'in rain-water which had stood but four days in a new earthen pot.'

\* G. N. Calkins ('The Protozoa,' 1901) recalls that Linnæus at first received Leeuwenhoek's discovery with complete scepticism, and only in the later editions of his work grouped protozoa, bacteria, etc., under the generic head of 'Chaos.'

The occurrence of protozoa wherever air and water are present is accounted for by a property that many of them possess when deprived of moisture; in these circumstances they shrink up and secrete a capsule (protection cyst) in which they suffer desiccation with impunity, and when thus dried they may retain their vitality for many years. In this state they are readily carried about as dust by movements of air. Those that chance to fall in water there resume their active state, and, if the conditions as to food, etc., are favourable, multiply apace. Thus arose the mistaken idea of 'spontaneous generation.'

Though many of the protozoa measure a millimetre or more in diameter, and hence are recognisable by the unaided vision, by far the greater number are of microscopic size; on the whole, they constitute a subdivision of the animal kingdom that is unseen, and hence is apt to be left out of consideration unless they are from time to time brought to our notice. The importance of the protozoa in terrestrial existence is evidenced by the fact that the chalk beds and cliffs and large areas of the ocean bed are formed by their accumulated skeletons. Their wide distribution is easily realized by watching the course of events in sterile aqueous infusions containing organic matter after they have been exposed to the air. First bacteria appear, and soon after them flagellate protozoa; a little later ciliated protozoa are found in increasing numbers.

The protozoa often present that striking degree of organization that led the earlier observers to regard them as being, save for their small size, similar in structure to the multicellular animals or *metazoa*. Parts of the unicellular body of a protozoon that possess a special

structure and function are termed 'cell-organs' or 'organelles,' to distinguish them from the compound cellular organs of the metazoa.

In the consideration of the protozoa in relation to disease it is not their function as earth-builders, etc., nor is it the anatomy of the more highly differentiated forms

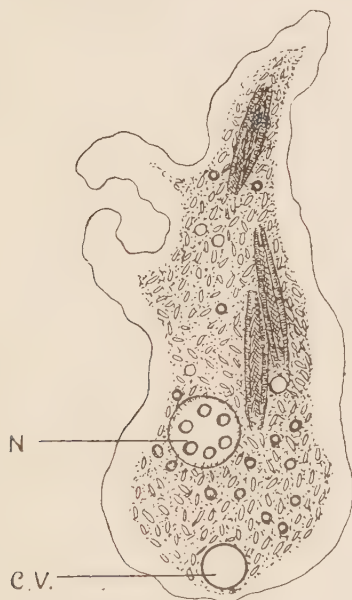


FIG. 1.—A COMMON FRESH-WATER AMOEBA SLIGHTLY SCHEMATIZED FROM A LIVING EXAMPLE.

The clear ectoplasm contrasts with the granular endoplasm which contains a nucleus, N, and a contractile vacuole, CV, besides three diatoms, fat-drops, and numerous oval granules.

that need detain us, but the essential structural features of the simpler forms in the varied phases that they assume in their life-cycle, and the modifications of known parasitic forms, must be most closely studied. The knowledge that has been accumulated in the investigation of the parasites of malaria affords an illustration of how

complicated both the life-history and the parasitic adaptation of protozoa may be.

The most natural way of renewing an acquaintance with the protozoa is to be found in studying typical examples of the various classes into which they are divided.\* Thus a common amœba (Fig. 1) may be taken as an

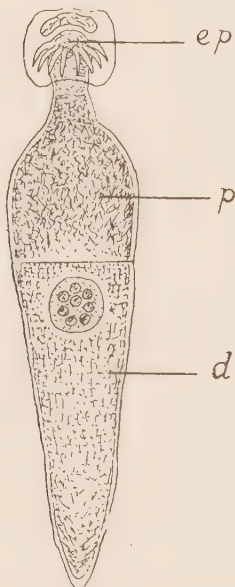


FIG. 2.—A GREGARINE FROM THE INTESTINE OF A COMMON CENTIPEDE. ( $\times 80$  diameters. From a stained preparation.)

The anterior extremity, *ep*, epimerit, carries hook-like appendages, and is still embedded in the host-cell. The hinder segment, *d*, deutomerit, contains the nucleus, whilst the intermediate segment, *p*, protomerit, is separated by a septum from the hinder one.

example of the *sarcodina* or *rhizopoda*; a common gregarine (Fig. 2) as an example of the *sporozoa*; a simple monad,

\* Delage and Hérouard, in their 'Zoologie Concrète,' vol. i. ('La cellule et les Protozoaires,' 1896), give a luminous insight into the protozoa by introducing each class by typifying composite examples which combine the salient features of each class and the chief subclasses of the protozoa.

*Euglena viridis* (Fig. 3), to illustrate the *mastigophora* or *flagellata*; and a paramæcium (Fig. 4) as an example of the *ciliata*. All are cells.

By direct light the organisms represented in Figs. 1, 2, and 4 appear whitish, owing to the refraction of light from the granules, etc., in their substance. The euglena has

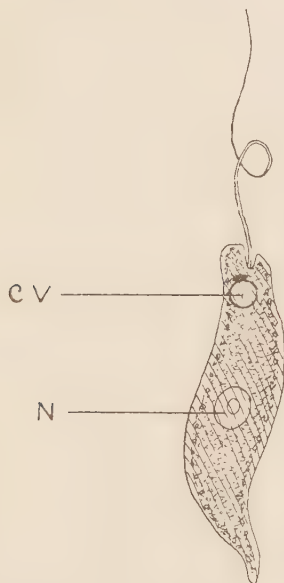


FIG. 3.—A COMMON FLAGELLATE, *EUGLENA VIRIDIS*.

The ectoplasm is obliquely striped, and beneath it are placed the chromatoplasts which contain chlorophyll. The flagellum is of about the same length as the animal's body, and at its base is the rudimentary pharynx. There is a contractile vacuole, CV, and close to this a pigment-spot. The nucleus, N, is near the middle of the body.

a green colour, by reason of the granules of chlorophyll situated beneath the cortical layer.

In considering diagrammatic pictures such as Figs. 1, 3, and 4, it has always to be borne in mind that they are summation drawings arrived at by adding together the

various features presented by the objects that they represent under various different circumstances. Thus, if we recall the very different aspects of a white blood cell: (1) as seen living in its natural active state, and looking by transmitted light like a faint bit of colourless, slightly granular jelly, with the nucleus either seen indistinctly, by

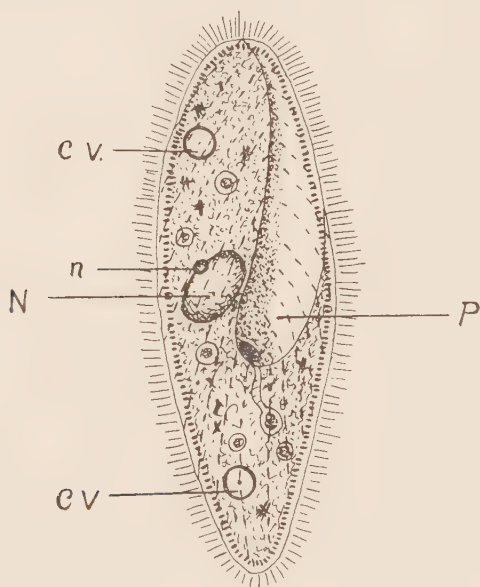


FIG. 4.—A COMMON CILIATE, PARAMÆCIUM.

The surface of the ectoplasm is evenly covered with cilia. To the animal's left is a large depressed area, P (peristome), leading to the mouth and pharynx. About the middle of the body and to the right is the nuclear body, consisting of two divisions: N the macronucleus and *n* the micronucleus. There are two contractile vacuoles, CV, and numerous food vacuoles, etc.

reason of its higher refraction-power, or totally obscured by granules; (2) the dead corpuscle as seen rounded, granular, and semi-opaque, in pus; (3) after the addition of weak acetic acid, with the granules dissipated, and the

nucleus showing sharply-defined in the clear protoplasm; and (4) after treatment by fixing reagents and stains:—in our conception of a leucocyte all these varying appearances are added together, forming in the mind a summation picture.

The above facts should be recalled, if only to accentuate the importance of examining all protozoa in the living or fresh state, as well as after the fixing, staining, and clearing processes necessary to make permanent preparations:—and this applies to examination of morbid tissues, with a view to finding protozoa in them.

*Parasitism.* — The main characteristics of parasitic adaptation on the part of animal organisms are so familiar—for instance, in the tapeworms, (*Filaria sanguinis hominis*, malarial protozoa, etc.—that they need not be dwelt on at length. A few words, however, may be said as to the essential characteristic of parasitism as exhibited by animals. It is illustrated by what is seen in a group of the *suctoria*, a subdivision of the *ciliata*. These protozoa possess cilia only in the earlier period of their life, and instead of a single mouth, they have a number of suckers, by means of which they can adhere to and suck the softer endoplasm from other protozoa. One of them, *sphærophrya*, is represented in Fig. 5. By means of its suckers it has captured six other ciliates, and constitutes a striking instance of predatory life. Some of the smaller varieties of the same order pierce the cuticle of their prey, and form brood-cavities in its interior. The young brood, escaping to the exterior, are ready to repeat the process (Fig. 6, *a, b, c*), which is typical of an endo-parasite. Essentially, the predatory and the parasitic life are the same.

Before passing to consider in some detail the structure of typical cells and protozoa, the question whether there exist non-nucleated forms of protozoa must be considered. Haeckel thought that such forms did exist, and he constituted them a class which he termed *monera*. Many of Haeckel's monera have been found by improved methods to possess demonstrable nuclei. There remain some forms in the earlier stages of which no nucleus can be demonstrated, and in them it is probable that the nuclear matter is interfused with the cytoplasm, and that the differentia-

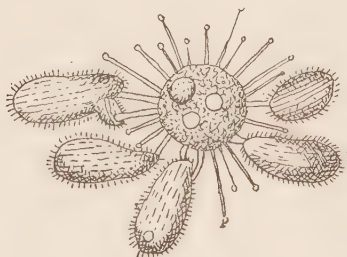


FIG. 5.—SPHÆROPHRYA MAGNA, A SUCTORIAN. (After Maupas.)  
The animal has captured six other ciliates ; in its interior the macro-nucleus and two contractile vacuoles are visible.

tion of cytoplasm and nucleus takes place only as the animal approaches maturity. These non-nucleated forms are of special interest with regard to the minute intracellular bodies that have been observed in small-pox and other diseases.

### THE CELL.

It is only by comparing the structure of cells familiar to us in human histology with that of such of the protozoa as are likely to be concerned in the causation of disease

that a basis for interpreting certain microscopic appearances in diseased tissues and fluids can be acquired.

A typical cell consists of cytoplasm and nucleus. The cytoplasm of most metazoan cells, as seen under the microscope, is more or less transparent. More highly magnified, especially after it has been coagulated and stained by suitable reagents, the cytoplasm is seen to possess some degree of structure, and though cytologists are not yet agreed

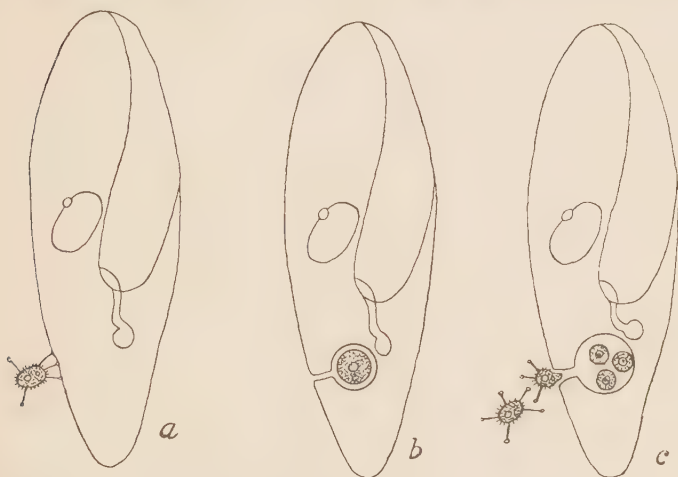


FIG. 6.—SUCCESSIVE STAGES IN THE EVOLUTION OF A SPHEROPHYRYA IN THE INTERIOR OF A PARAMÆCIUM; THE LATTER IS SHOWN IN OUTLINE ONLY. (Modified from Delage and Hérouard.)

*a*, Young parasite attached to a paramæcium; *b*, the parasite has perforated the cuticle, and is lodged in a cavity in the endoplasm; *c*, the parasite has broken up into swarm-spores, some of which are escaping to the exterior.

upon the interpretation that should be put upon all the appearances of formed parts to be observed in the histological examination of cells, nearly all recognise a fibrillar and a clear non-fibrillated part of the cytoplasm, These are termed respectively the *spongioplasm* and the

*hyaloplasm*. Other formed elements are granules and vacuoles. The *granules* are usually minute—e.g., about  $1\ \mu$  in diameter—and of variable shape and size (Fig. 7). In the secreting cells of certain glands granules of larger size are present. Certain stains, such as acid fuchsin, render the granules more evident, and bring into view many that were previously invisible.

The *vacuoles* are cavities in the hyaloplasm, and probably contain aqueous solutions of salts and albuminous matters.

*Cell-membrane*.—The outer surface of the cytoplasm is frequently condensed into a firmer layer, the cell-mem-



FIG. 7.—DIAGRAM OF A CELL.

The outer circle represents the cell-membrane, the inner the nuclear membrane. The lower segment of the cell-protoplasm has been represented in detail, showing reticulum of spongioplasm containing in its meshes vacuoles and granules. Above the nucleus is the centrosome, with attraction-sphere and aster. The nuclear network and a nucleolus are shown.

brane. This in some cells is thickened into a definite cuticle. The intercellular substances, such as the fibrils of connective tissue, may be regarded as special developments of the cell-membrane.

The *nucleus* tends to have a rounded or 'vesicular' form, and to occupy the middle part of the cell. It is limited by a membrane, and its bulk (enchylema) is of liquid

character, not gelatinous, as is the case with the cytoplasm. The vesicular form is, however, often widely departed from, as in the multipartite nucleus of the common polymorphous leucocytes.

The *nuclear membrane* is a delicate structure that is distended by the pressure of the enchylema. It disappears during a short phase of division of a cell, to be re-formed at the end of that period.

The *enchylema* or nuclear juice is formed of a watery solution of salts, with a trace of albumin.

*Linin*.—The formed elements of the nucleus appear to consist of a network which does not take the ordinary stains, and is termed *linin*, which is comparable to the spongioplasm of the body of the cell.

*Chromatin*.—Upon, or sometimes within, the fibrils of linin are deposited minute grains of a substance which stains readily, and hence is termed chromatin.

*Nucleoli*.—Free in the nuclear juice there may be one or more bodies that react to stains, but often in a slightly different manner to the chromatin. During indirect division of the cell the nucleoli diminish in size, and usually disappear, to be re-formed in the later stages of division. These true nucleoli or *plasmosomes* are to be distinguished from agglomerations of chromatin. In some cells they are entirely absent; when present, they vary in number from one to five. In the eggs of some lower vertebrates there are hundreds of nucleoli. In the subcutaneous gland-cells of *pisciola* the originally single nucleolus subdivides into several hundreds, all *but one* of which migrate into the cytoplasm.\* The nature of true nucleoli is still imperfectly known.

\* Wilson, 'The Cell in Development and Inheritance,' 1900.

*Indirect Division of Cells.*

The most important reproductive process in cells composing the bodies of the metazoa is that known variously as indirect division, karyokinesis, or mitosis. During this function the centrosome and other cell-organs play an important part.

The *centrosome* is usually seen only during indirect cell-division. In the resting stage it is lodged in the cytoplasm, near one part of the nuclear membrane, or sometimes in the interior of the nucleus. When in the latter position it has been confused with the nucleolus, but since both nucleolus and centrosome have been observed to persist throughout the whole period of division, their independence has been established.

During the spirema stage of nuclear division the centrosome becomes marked out by a radiating disposition of the surrounding protoplasm, the fine radiating striæ constituting the *aster*; between the origin of the striæ and the centrosome a hyaline zone of protoplasm, the *attraction-sphere*, is formed. Soon after the differentiation of these parts the centrosome elongates and divides into two minute bodies, which separate, each surrounded by its own attraction-sphere. Between the two new centrosomes fine achromatic striæ form a small spindle.

During indirect division of a cell the network of linin, with the adherent (or contained) chromatin, separates first in a long spiral filament (spirema), which subsequently becomes shorter and thicker and finally breaks into *chromosomes*, which in animal cells usually number from twelve to twenty-four. The dissolution of the nuclear membrane\* begins on the deeper aspect of the small

\* Cytologists are still undecided as to what becomes of the cell-membrane and the nucleoli when they disappear during mitosis.

spindle uniting the two chromosomes, which continue to recede from each other, the original aster now subdivided into two, which constitute the *antipodal cones* (Fig. 8, *a c*). Finally, from each attraction-sphere a set of achromatic

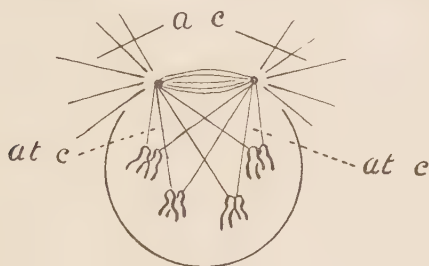


FIG. 8.—DIAGRAM OF THE CHIEF PARTS CONCERNED AT A STAGE IN INDIRECT CELL-DIVISION.

*a c*, Antipodal cones ; *at c*, attraction-cones.

striæ pass to the chromosomes (now dividing into halves) and constitute the *attraction-cones* (Fig. 8 *at c*), which, when the divided chromosomes recede towards the poles of the nucleus, constitute the *peripheral spindles* (Fig. 9, *p.s.*).

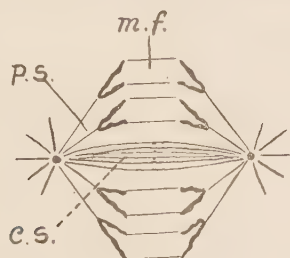


FIG. 9.—DIAGRAM OF A FURTHER STAGE OF REGULAR MITOSIS.

*p.s.*, peripheral spindle ; *c.s.*, central spindle ; *m.f.*, mantle-fibres.  
The nuclear membrane has disappeared.

The original small spindle joining the centrosomes becomes elongated and forms the *central spindle* (Fig. 9, *c.s.*), and connecting the separated halves of the chromosomes fresh achromatic fibres, *mantle fibres* (Fig. 9), appear.

The subsequent details of indirect cell-division need not be detailed here, but in order to apprehend the differences exhibited by dividing tissue-cells as compared with corresponding processes in various protozoa the points detailed above are to be borne in mind.\*

Indirect cell-division does not always occur in the manner indicated above. In a second mode of nuclear division the centrosome divides and the segments pass to the poles of the nucleus without the formation of a central spindle; the attraction-cones arise in the perinuclear

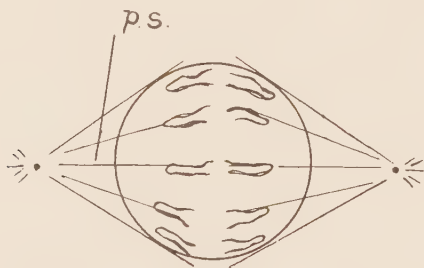


FIG. 10.—A SECOND FORM OF MITOSIS.

The peripheral spindles, *p.s.*, and centrosomes are present, but there is no central spindle.

plasma, the chromosomes becoming attached to the rays of the attraction-cones (Fig. 10). The nuclear membrane gradually disappears as the process of division advances.

*Pathological Mitoses.*—In rapidly proliferating pathological tissues, especially cancer and sarcoma, many of the mitotic figures are abnormal. Some present an excess, some a deficiency of chromatin; others are exaggerated

\* Whilst, as above stated, the centrosome and associated structures are only plain during nuclear division, in certain cells, such as the leucocytes of the salamander, they are visible during the resting stage of the nucleus, as has been shown by M. Heidenhain ('Ueber Kern und Protoplasma') in Kölliker's *Festschrift*, 1892, from which Fig. 11 is taken.

in size (giant mitoses). Others, again, are atypical; instead of the usual bipolar arrangement, the daughter centrosomes may be placed near together, or they may be multiple, as many as five centrosomes being present in the multiple mitotic figure. In other cells the mitosis has the appearance of being abortive—checked in its course and otherwise altered, or accompanied by what are regarded as degenerations in the nuclear or cytoplasmic elements. Some of these appearances have been interpreted as parasites, whether protozoa or protophyta; they will be discussed later in this work. The interpretation of them that is at present held by most pathologists has been clearly stated by Pianese.\*

#### *Direct Cell-Division or Amitosis.*

In typical direct cell-division the nucleus, without any recognisable rearrangement of chromatin akin to mitosis, becomes elongated in one direction, constricted in the middle, and finally separated into two equal parts. The cytoplasm does the same, and the act of division is thus completed. This form of cell-division was formerly the only one known. In the follicle cells of the mole-cricket Carnoy found that the division begins in the nucleolus, and is followed by that of the nucleus. Arnold described various modes of direct cell-division.

#### *Cells of Endogenous Origin.*

One of the questions that will be discussed in the following section dealing with malignant growths is whether the explanation first given by Virchow of cells

\* G. Pianese, 'Zur Histologie des Carcinomes,' supplement to Ziegler's *Beiträge*, 1896, pp. 67-95.

that occur in the cytoplasm of certain other cells in cancer, sarcoma, etc., can be explained by a process of endogenous formation similar to that by which the white and red blood cells arise in the cells of bone marrow, etc. In the present place the description of the origin of colourless corpuscles in certain cells of bone marrow, as described by Denys,\* may be briefly quoted.

The hæmatopoietic cells of marrow are distinct from



FIG. II.—A LEUCOCYTE OF A SALAMANDER, SHOWING A WELL-DEVELOPED CENTROSOME AND ASTER, WITH A RESTING NUCLEUS. (After M. Heidenhain.)

the osteoclasts. They are of two kinds: In one the nucleus is of clear appearance, possessing a nuclear membrane, nuclear juice, filaments of chromatin and nucleoli (Fig. 12), the two latter only reacting to stains. In the other the nucleus is dense, and the network of chromatin is either indistinguishable or is seen only in a portion of the nucleus (see Fig. 13). The cytoplasm is

\* J. Denys: 'Cytodierèse des Cellules Géantes et des petites Cellules Incolores de la Moëlle des Os.'—'La Cellule,' vol. ii., 1890.

sometimes of the ordinary type, slightly granular and not very transparent, and sometimes it has a dark appearance owing to the presence of a great number of fine granules. All gradations between the two kinds are met with. In

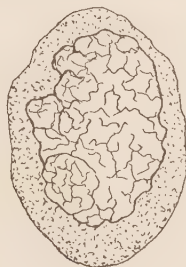


FIG. 12.—A CELL OF THE RED BONE-MARROW OF A RABBIT, WITH CLEAR RETICULAR NUCLEUS. (After Denys.)

the marrow of the rabbit projecting portions of the nucleus of the hæmatopoietic cells are constricted off, and portions of the cell-protoplasm surrounding these detached nuclei separate from the rest, forming typical cells



FIG. 13.—ANOTHER CELL OF THE RED BONE-MARROW OF A RABBIT, WITH DENSE NUCLEUS ONLY IN PART RETICULAR. (After Denys.)

of endogenous origin. These newly formed cells either escape singly from the mother-cell or are liberated simultaneously by disintegration of the remains of the mother-cell (see Fig. 15).

Sometimes the same end is attained by a kinetic pro-

cess (Fig. 16). Thus identical cells are produced indifferently by segmentation or kinesis, supporting Carnoy's



FIG. 14.—ANOTHER CELL FROM THE SAME SOURCE. (After Denys.)

Portions of the nucleus have been separated by constriction and new endogenous cells formed.

view that the two forms of cell-division are not essentially different. It will be seen later how closely the mode of

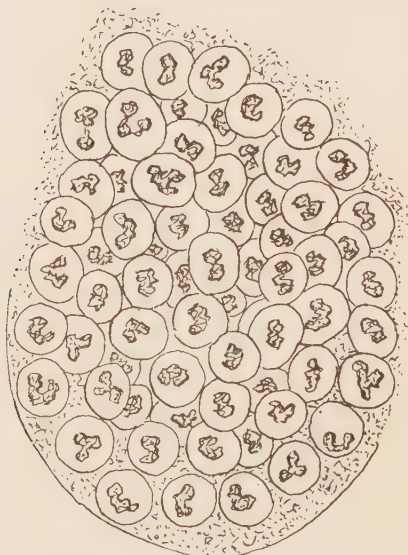


FIG. 15.—ANOTHER MARROW-CELL, IN WHICH A BROOD OF ENDOGENOUS CELLS HAS BEEN FORMED. (After Denys.)

reproduction of some of the protozoa resembles that of these cells of the marrow.

*Wandering Cells.*

The amœboid and phagocytic character of leucocytes and other wandering cells, and the attraction that certain substances possess for them (chemiotaxis), causes accumulations of these cells in various conditions of inflammation and new growth, with the result that appearances such as that shown in Figs. 14 and 15 may arise from a totally different cause.

*The Protozoan Nucleus.*

The four examples of protozoa given above all possess nuclei. That of the amœba (Fig. 1) presents a nuclear membrane and nuclear juice. The solid constituents of

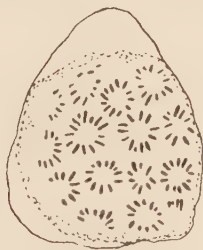


FIG. 16.—A MARROW-CELL, IN WHICH NEW CELLS ARE BEING FORMED BY A KINETIC PROCESS. (After Denys.)

the nucleus are simply masses of chromatin in which neither chromosomes nor nuclei can be distinguished. The nucleus of the gregarine (Fig. 2) is similar to that of the amœba. In the flagellate euglena (Fig. 3) there is within the nucleus a body that might be taken to be a nucleolus, but which has been proved to have the same function as the centrosome of the metazoan nucleus as described above; it is an internal division-centre or centronucleus. The nucleus of the ciliate (Fig. 4) is what is termed dimorphic. Its larger division, the 'N,' or

macronucleus, is functional chiefly in vegetative reproduction, whilst the 'n,' or micronucleus, is more especially concerned in conjugation. With one doubtful exception among the flagellates, dimorphic nuclei are limited to the infusoria. The macronucleus in some genera assumes various shapes; in others, such as trachelocerca (Fig. 17), the nucleus consists of chromatin granules scattered widely through the cell.

From such a nucleus it is a short step to a condition in



FIG. 17.—A CILIATE, TRACHELOCERCA. (After Wilson and Gruber.)

Its nucleus consists of scattered chromatin granules.

which nucleus and cytoplasm are completely intermingled throughout the whole cell (see above, p. 8); conversely the nucleus in some protozoa consists of a single mass of chromatin of homogeneous appearance. In some of the protozoa extranuclear division-centres, closely comparable with the metazoan centrosome, are present. This arrangement is shown in Fig. 18.

*The Protozoan Nucleus in Division.*—In many of the lower amœbæ direct division appears to prevail ; in others

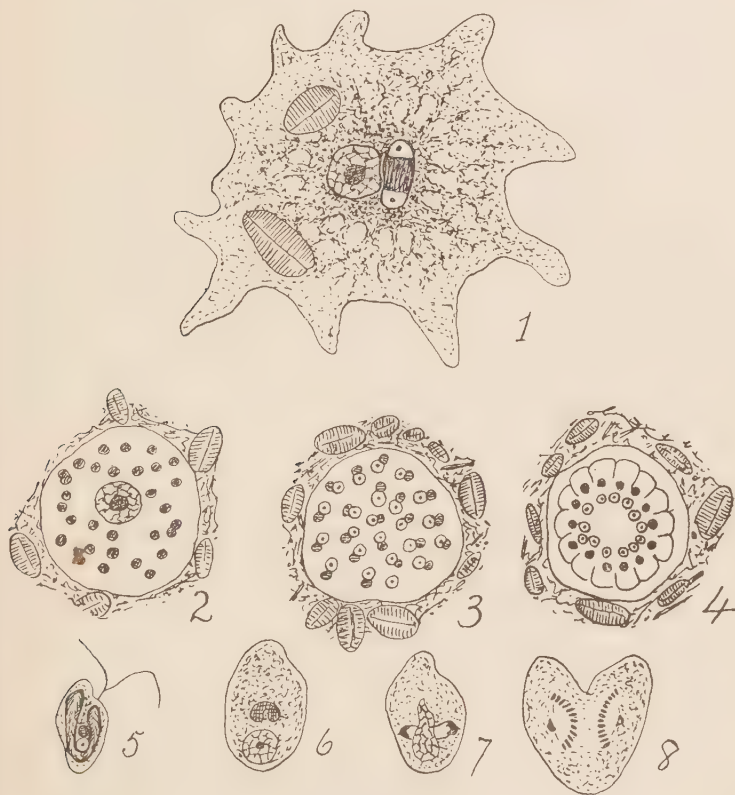


FIG. 18.—PARAMŒBA EILHARDI. (Schaudinn.)

1, The amœba with nucleus and extranuclear division-centre (Nebenkörper) ; 2, central part of the same at the beginning of sporulation, the division-centre is subdivided, the nucleus remains unchanged ; 3, the nucleus has subdivided, and the divisions have attached themselves to those of the division-centre ; 4, commencing segmentation, which terminates in the formation of flagellate swarm-spores ; 5-8, stages in the division of one of the swarm-spores.

a rudimentary mitosis without centrosome, but with an imperfect spindle, is seen. In the gregarines, as in other

sporozoa, the nuclear processes are complex. The formation of numerous spores is preceded by successive nuclear divisions with well-marked spindles.

In some of the higher flagellates—e.g., *noctiluca*—an extranuclear division-centre is present, whilst in others, like *euglena*, already referred to, the division-centre is within the nucleus. In the ciliata both 'N' and 'n' divide with a simplified mitosis, and in some forms there has been observed in the 'N' a division-centre which consists of a central granule surrounded by an achromatic substance. In some of the suctoria there is a much branched nucleus, either from the ramified form of the animal or from numerous offshoots from the nucleus being formed in budding. Such branched nuclei closely resemble irregular nuclear forms that occur in certain sarcomata. During spore formation intricate nuclear processes occur in many sporozoa, as, for instance, in the rhizopod *paramœba* which presents the curious alternation of generations shown in Fig. 18.

*Sexual Processes in Protozoa.*—Recent researches have shown that in the coccidia, including the malarial parasites, sexual processes closely akin to those of the metazoa occur. Formation of male elements which correspond to spermatozoa, maturation of the female cell by the extrusion of nuclear matter and fusion of the male with the matured female element, all take place, as in the metazoa, and are accompanied by complicated nuclear changes, which will be detailed below.

## CHAPTER II

### THE SARCODINA

THE class of the protozoa to which the amœbæ belong are variously named rhizopoda, sarcodina, or gymnomyxa. The latter term, introduced by Ray Lankester, refers to the absence of a cuticle; for though many of the higher orders of this class—the foraminifera and radiolaria—possess complicated skeletons, external or internal, some part of the body surface is always naked protoplasm. The lower orders of the class deserve especially close attention.\* These are the two subclasses, the proto-myxiæ and the mycetozoariæ.

1. *Proteomyxiæ* (Ray Lankester).—Lowly organized forms which farther study may show to belong to other orders, include the following orders:

(a) The *Acystosporida*, naked amœbæ, often devoid of a demonstrable nucleus, with reticulated pseudopodia, and without any differentiation between ectoplasm and endoplasm, reproduction being by direct division without encystment. The *Protogenes primordialis* of Haeckel is an example.

(b) The *Azoosporida*, which rarely lack a nucleus, and which sometimes possess a contractile vacuole. *Vampyrella*, a simple amœba which lives as a parasite on the

\* The account given by Delage and Hérouard is followed here.

lower algæ, is a typical example. It has a hyaline ectoplasm, a granular endoplasm, nucleus, and pulsatile vacuole. It puts out delicate pseudopodia, and by means of some of these it perforates the cell-wall of an alga and absorbs its protoplasm and chlorophyll (Fig. 19). Its principal mode of multiplication is as follows: The animal becomes encysted and subdivides into a number of small protoplasmic masses which escape from the cyst by piercing its walls, and assume immediately the amœboid characters of the parent. Reproduction by simple division of the

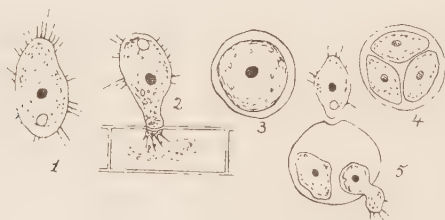


FIG. 19.—VAMPYRELLA. (After Delage and Hérouard.)

- 1, The animalcule with fine radiating pseudopodia, nucleus, and contractile vacuole; 2, a vampyrella that has pierced the cell-wall of an alga and pushed pseudopodia into its substance; 3, encysted form; 4, division after encystment; 5, the young brood escaping after piercing the cyst-wall.

whole parasite also occurs. The animalcule generally has a reddish coloration, it measures from 5 to 7 millimetres, and lives in both fresh and salt water.

(c) *The Zoosporida*.—Small amœbæ, usually nucleated, rarely provided with a pulsatile vesicle, living like the preceding as parasites on the lower algæ (diatoms, spirogyra, etc.). When two or three are together within the same host-cell they may fuse together into a *plasmodium*. On attaining maturity the parasite withdraws

its pseudopodia and becomes encysted, and divides into a number of nucleated cells and a residual unsegmented body. The characteristic feature of the order is that the young as they escape from the cyst are provided with a single flagellum—*i.e.*, they have the aspect of small monads, to which the ill-suited name of *zoospores* has been given. The flagellum, unlike that of the true monads, is not distinct at its base, but is gradually prolonged into the pyriform body of the animalcule; the flagellum is, in fact, only a modified pseudopodium, which does not greatly subserve locomotion, causing, rather, agitation without any marked progression. When the parasite reaches its host-cell it becomes attached to and pierces the cell-wall, losing its flagellum, and lives on the contents of the host-cell, the substance of which it may enter wholly or only partially. Some of the zoosporida were previously included with the Synchytriaceæ, a parasitic group of the lower fungi; they have been shown (especially by W. Zopf\*) to be protozoa.

One interesting example, *Plasmodiophora brassicæ*, at present included with the zoosporida, causes a disease in various cruciferous plants. In the following description it will be observed that *P. brassicæ* differs from other zoosporida in that subdivision into spores is not preceded by encystment; its parasitic habit may account for this divergence.

*Plasmodiophora Brassicæ* (Woronin†).—This parasite

\* W. Zopf, 'Ueb. einige niedere tierische und pflanzliche Organismen, Welche als Krankheitserreger in algen, Pilzen, niederen Tieren und höheren Pflanzen auftreten,' *Beit. zur Physiol. u. Morphol. niederen Organismen*, Heft 4, pp. 43-68, 1894.

† M. Woronin. *Jahrbücher für Wiss. Botanik*, vol. ii., 1877-78 Quoted by Doflein, 'Protozen als Parasiten,' etc.

causes irregular lumpy growths, known in this country as 'finger and toe,' on the roots of several plants of the cabbage tribe—cabbage, beet, etc. In certain localities the parasite has caused serious damage; the greater number of the infected plants are destroyed by the parasites, and those that are not killed outright are made useless for food. The appearance of the diseased

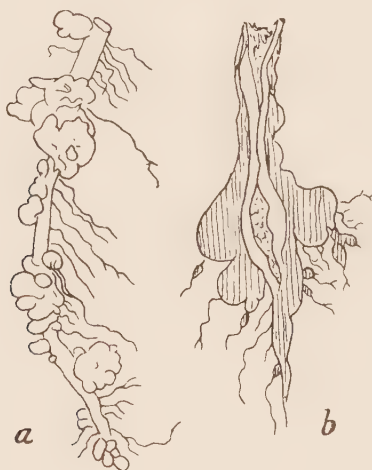


FIG. 20.—ROOTLETS OF CABBAGE INFECTED BY THE PLASMODIOPHORA BRASSICÆ.

*a*, Surface view, after Woronin; *b*, section, after L. Pfeiffer.

rootlets is shown in Fig. 20, *a* and *b*. The spores appear to remain dormant in the earth during winter, and in the summer infect the next crop of plants. One section, the cortical layer, of the root is seen to be irregularly thickened and of an opaque gray colour. In sections of the infected tissue masses of spores are readily recognised under the microscope. If a slice of one of the tumours is placed in distilled water for one or two days

and again examined, it has been found that most of the spores have escaped, and they are found floating in the water. The young parasite escapes from the spore as a slender amœboid organism provided with a motile flagellum at its anterior extremity (Fig. 21). These active organisms enter the cortical cells of the root, and there they assume an amœba form, the flagellum disappearing. The host-cells possess a peripheral layer of protoplasm, which is joined to the nucleus in the middle of the cell by protoplasmic bridges, the intervening spaces being filled with fluid sap. The youngest parasites are difficult to distinguish from the protoplasm of the host-



FIG. 21.—THE SPORE OF *PLASMODIOPHORA BRASSICÆ*, AND THE ESCAPE OF THE YOUNG PARASITE THEREFROM.  $\times 620$ .

cell. After fixation with osmic acid the parasites, most of which contain two or more nuclei, are seen to be filled with blackened granules. The nuclei of the parasite consist of a nuclear membrane, a large nucleolus and a network of chromatin (Doflein). Feinberg\* describes the nucleus as follows: 'The nucleus of the adult amœba and of the young parasite consists of a nucleolus, and outside this a clear, unstained zone which is separated from the cytoplasm by a sharp boundary.'

Feinberg's statement that the nuclei of all protozoa have these characters is incorrect. As the parasites

\* Feinberg, *Deutsch. Med. Woch.*, January 16, 1902.

grow the nuclei increase in numbers by a simple form of karyokinesis. The parasites appear also to divide by constriction without any accompanying nuclear division. From the continued absorption of the substance of the host-cell the parasites occupy more and more of the cell cavity, and they then fuse together into a plasmodium, the nuclei of which undergo simultaneous karyokinetic

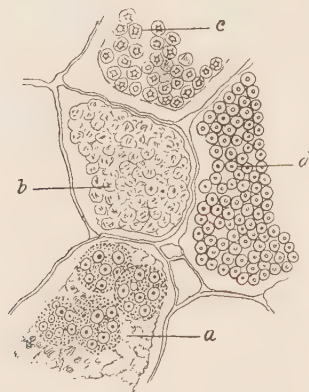


FIG. 22.—PART OF A SECTION OF AN INFECTED CABBAGE ROOTLET.  
(Modified after Doflein and Woronin.)

- a*, Four multinucleated amoeboid parasites in a cabbage-cell; *b*, plasmodium occupying almost the whole of the host-cell and undergoing nuclear subdivision; *c*, early stage of spore formation; *d*, spore formation completed.  $\times 400$ .

subdivision, the whole plasmodium being crowded by minute nuclei (Fig. 22, *b*), devoid of nucleoli, and many of them of indefinite form. Finally, the protoplasm divides into segments, each of which contains a nucleus and secretes a membrane, thus constituting a spore (Fig. 22, *c* and *d*). The host-cell now contains nothing but these spores and a few starch grains and detritus. The parasite lives by osmosis of the cell-sap. From the protoplasm of

the host-cell a fine capsule may be formed around the parasites. Many of the infected cells undergo mitotic subdivision. The parasites are liberated by the decay of the infected roots. As prophylactic measures Woronin recommends burning all roots after the crop has been secured, an alternation of crops, and careful selection of young plants.

2. *Mycetozoa* are amœbæ that fuse together temporarily or permanently in the course of their life into colonies or plasmodia. They are divided into the

- (a) Pseudoplasmodida.
- (b) Filoplasmodida.
- (c) Euplasmodida, or Mycetozoa.

The *Pseudoplasmodida* are small amœbæ with nucleus and contractile vacuole, few and relatively large pseudopodia. After a period of individual life in which they undergo numerical increase by successive divisions, the parasites fuse together in small masses, and secrete a gelatinous material, which envelops them. The individual parasites become encysted ( $10\ \mu$ ), and under favourable conditions escape from their single or double capsules and disperse. Found on horse-dung, etc.

*The Filoplasmodida*.—Of these, labyrinthula (Fig. 23) is the best-known example. These small amœbæ are always united together by their filamentous pseudopodia into colonies. They move by contraction of the uniting filaments. Numerical increase is by transverse division. Encystment may occur individually or collectively. The former is an encystment of repose, the latter is reproductive.

*Euplasmodida*, or *Mycetozoa*, live on dead leaves, wood,

etc. The best-known example is the 'flowers of tan,' found on the oak-bark in tan-pits. The adult amœba is a simple lobed and nucleated amœba. They multiply rapidly in favourable, whilst in unfavourable surroundings they secrete a cellulose cyst-wall. When nourishment becomes scanty they fuse together, forming plasmodial masses, which may be several centimetres, or even decimetres, in width. The composite animal moves and lives as a large amœba, and may become reticulated from adapting itself to obstacles. The individual amœbæ may become encysted,



FIG. 23.—*LABYRINTHULA CIENOWSKYI*, ONE OF THE *FILOPLASMODIDA*, INVADING A CELL OF A FILAMENTOUS ALGA.

‡The cell-contents of the alga are omitted.

and so remain dormant for years. Regular encystment occurs by the plasmodium as a whole becoming rounded off and, usually, elevated on a kind of stalk, a specialized part of the cellulose sporangium, within which the individual amœbæ also become each surrounded by a cyst of cellulose. The mycetozoa were formerly termed 'myxomycetes,' or slime-fungi. Most zoologists are now of accord in regarding them as animals.

'The appearance of cellulose was the only justification for regarding these animals as in any way allied to plants, and it is known that cellulose is quite a constant product in some groups of animals.'\* Calkins† suggests that this

\* Shipley and Macbride, 'Zoology,' 1901.

† Calkins, 'Protozoa,' p. 18.

subclass may constitute a link between animals and plants, and belong to both kingdoms. In the amœboid stage certain mycetozoa incept and digest solid food such as yeast-fungi. Their life-cycle (see Fig. 24) is much the same as that of the *Plasmodiophora brassicæ* described above, only in the latter the encystment of the plasmodium as a whole is wanting, probably, as Delage and Hérourard



FIG. 24.—DIAGRAM TO ILLUSTRATE THE MAIN FEATURES OF THE MYCETOZOA.

- 1, Escape of amœba from the spore ; 2, the young parasite with flagellum, nucleus, contractile vacuole ; 3, later stage of individual organism, the flagellum retracted ; 4, the organism within a protection-cyst ; 5, complete fusion of many organisms into a plasmodium ; 6, (on a smaller scale), formation of sporangium.

suggest, because the protection afforded by the host-cell is sufficient in the parasitic species.

#### AMÆBINA.

The amœbæ proper occur, some in fresh water, many in the sea, a few in the soil, and they are by no means uncommon as parasites—*e.g.*, the well-known *Amœba coli*, sometimes found in the stools and the pus of abscesses in tropical dysentery.

The different species vary in size from 30 to 500  $\mu$ . They differ from the allied orders in that the amœba form constitutes their highest stage in development. The general characters of an amœba have been already referred to (p. 3). The degree of fluidity possessed by the drop of protoplasm that constitutes the animal's body varies in different species. Thus the *Amœba blattæ*, found in the intestine of the cockroach, is a relatively tough form, whilst *Amœba proteus* is more fluid.

The general structure and division of the body-substance into ecto- and endo-plasm has already been mentioned. By suitable histological methods an alveolar meshwork is seen to constitute the basis of the protoplasm. The ectoplasm is more highly refracting, tenacious, and hyaline than the endoplasm, which is rendered opaque by the various granules, vacuoles, and remnants of food that it contains. In some forms chlorophyll granules, starch, and fat drops occur.

*Nutrition.*—In view of the established method of investigating bacterial diseases by pure cultures, it is important to remember that the amœba feeds upon solid particles, which the pseudopodia surround and thus transfer to the interior of the animal, where they are digested in the endoplasm, any indigestible remains being rejected by being simply left behind in the flowing movement of the animal. Algæ and bacteria form the chief food of the commoner fresh-water amœbæ.

*Modes of Reproduction.*—The commonest of these, both in naked and in shelled amœbæ, such as *arcella* and *diffugia*,\* is simple transverse division. In the shelled

\* Both occur in the mud of ponds.

forms part of the body projects from the orifice of the shell for the pseudopodia ; after division of the nucleus the projecting part of the body secretes a new shell, and thus the two-shelled amœbæ result. At first they adhere together. The process is rather a modified budding than a simple transverse division. The nucleus in some species divides directly, in others mitotic division has been observed.

*Spore Formation.*—In *Amœba proteus*, besides the simple division, multiple division after encystment has been observed.

The life-cycle of a typical amœba may thus be summarized in a diagram such as Fig 25, but it should be remembered that the whole of the processes therein represented have not been observed in all the best-known species, and the diagram thus represents a summation of phases observed in different species.

One amœba, *Paramœba eilhardi*, presents a life-cycle complicated by an alternation of generations: (1) An amœba generation, in which multiplication both by transverse division and spore-formation is observed; and (2) a flagellate generation with multiplication by longitudinal division occurs. The nuclear processes exhibited by this amœba are particularly noteworthy. The nuclear body consists of a nucleus and a paranucleus, or *nebenkörper*, as it is termed by Schaudinn. This body resembles a centrosome in its behaviour. After a term of vegetative existence, marked by multiplication by transverse divisions, spore-formation takes place as follows: The paramœba first extrudes the diatoms that it has engulfed, withdraws its pseudopodia, and, becoming rounded, begins to rotate, and secretes a gelatinous

envelope, and within this a membranous capsule. The rotation ceases when the capsule is completed. These processes occupy a period of five days.

Thus encapsuled, the remarkable form of nuclear segmentation (described above, p. 21) occurs. The young

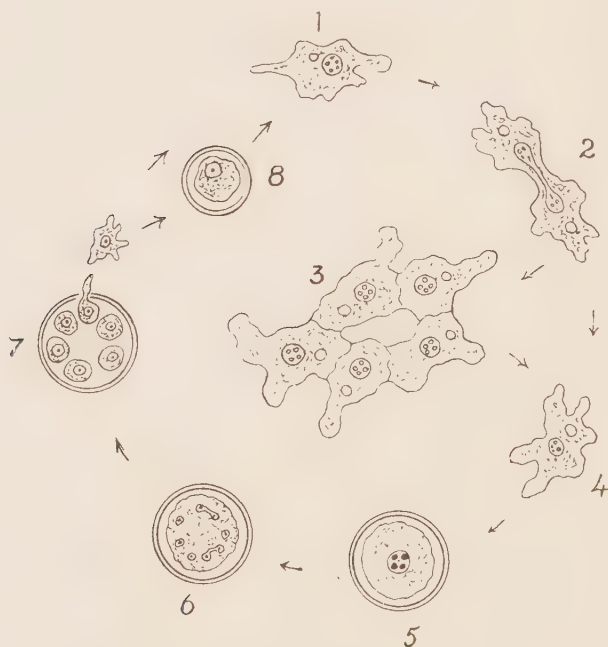


FIG. 25.—DIAGRAM ILLUSTRATING THE LIFE-CYCLE OF THE AMŒBINA.

- 1, Adult state ; 2, subdivision ; 3, association (plasmodium formation) ; 4, individual resulting from the breaking up of a plasmodium ; 5, encystment ; 6 and 7, subdivision within a cyst, and escape of young amœba ; 8, protective encystment.

escape in the form of flagellated bodies (Fig. 18), which multiply further by longitudinal divisions. In this they differ from zoospores, which either directly or after con-

jugation develop into the original form. The brood of flagellated paramœbæ must thus be regarded as a generation. Each flagellate has a cell-pharynx and a brownish chromatophore. The division of the *nebenkörper* precedes that of the nucleus. After a series of such divisions the

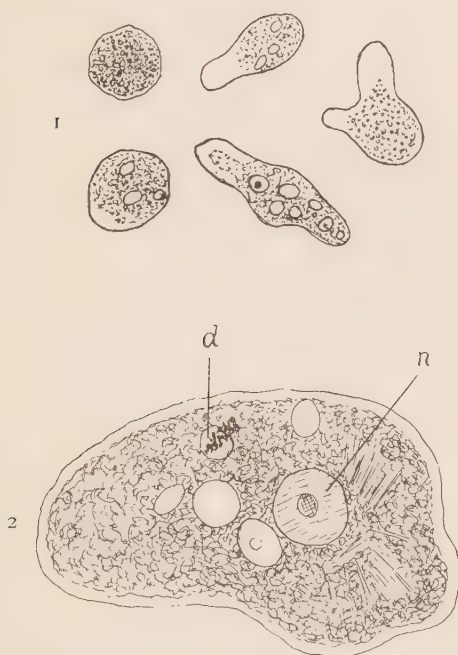


FIG. 26.—AMŒBA COLI.

1, As seen in the fresh state (after Lösch); 2, as seen after fixation in Flemming's fluid and staining with picro-carmin. The nucleus (*n*) and some pigment (*d*) from the digestion of red blood corpuscles are shown (after Councilman and Lafleur).

individuals of the flagellate generation fall to the bottom of the water in which they live, and their flagella and chromatophores disappear, pseudopodia appear, and the amœba generation is thus begun.

*Amæba Coli.*

In the subclass amoebina many species occur that inhabit the intestinal canal of different animals—*e.g.*, the *Amæba blattæ* in the intestine of the cockroach; the *A. ranarum* in the frog; an amoeba that occurs in rats and mice, *A. muris*, closely resembles the *A. coli*, which is more familiar, owing to its occurrence in some forms of dysentery in man. First described by Lösch\* in 1875, many observers have confirmed and added to the original description. The animal varies in size from 7.5 to 50  $\mu$  in diameter. The ectoplasm is slight in amount, and is only readily visible where a pseudopodium is beginning to form. The endoplasm contains a nucleus and several vacuoles. None of the latter have been found to possess the characters of a contractile vacuole. Granules and incepted food in the form of red and white blood corpuscles and bacteria also occur. Under favourable conditions the movements of the pseudopodia are active. The pseudopodia are few in number, usually one or two, and they are bluntly lobose in form. The nucleus is vesicular, and contains a nucleolus (see Fig. 26). With hæmatoxylin and eosin Jaeger† found that the nucleus stained a red colour.

Two modes of reproduction have been described in *Amæba coli*. Kruse and Pasquale found double forms, which they took to indicate a process of simple division. Grassi observed cysts that contained multiple nuclei, and regarded this as indicating a process of multiple division such as is familiar in other forms of amœbæ; and, though Grassi's view has been opposed by other observers, it may

\* E. Lösch, *Virch. Archiv.*, vol. lxx., p. 196, 1875.

† H. Jaeger, *Cent. für Bakt.*, May, 1902.

well prove, as Doflein observes, that there is a twofold mode of reproduction, and that the amœbæ within their host increase in numbers by simple divisions, whilst multiplication within cysts occurs outside the body, and subserves the spread of the parasite to other hosts.

As to the part played by this amœba in the production of certain forms of dysentery different views are held. Some authors regard the presence of the parasites as an accidental epi-phenomenon, whilst others regard them as the causal agent in some forms of dysentery. There can be no doubt that the swarms of amœbæ that invade the tissues of the intestines and the liver substance in the walls of abscesses must aggravate the intensity of the disease. The following observations of Patrick Manson\* are well worthy of consideration, as touching the significance of the concurrence of liver abscess and amœbæ in the absence of bacteria in certain cases of dysentery.

‘In a large proportion of liver abscesses the usual pyogenetic bacteria are absent. This has been proved over and over again. Cultures made with such pus often remain sterile. It is a very singular coincidence that it is just in those forms of suppurative hepatitis in which the usual pyogenetic organisms are absent that we find this other parasite present. Moreover, a liver abscess is not like an ordinary abscess: it has no proper abscess wall. Liver pus is not like pus elsewhere: it contains proportionately very few pus corpuscles, but it contains much tissue débris, many blood-cells, and much granular matter. As an abscess it is altogether peculiar. A peculiar effect suggests a peculiar cause. Anyone who has watched the movements of the *Amœba coli* on the warm

\* Manson, ‘Tropical Diseases,’ pp. 33 and 393, ed. 1900.

stage can readily understand how such an organism might break down and separate the anatomical elements of an organ like the liver, and so cause a softening—a cavity resembling an abscess. . . .

‘According to my experience of tropical abscess of the liver seen in England, *Amœba coli* can be detected in considerably over half the cases. This agrees with Kartulis’s experience in Egypt and that of others elsewhere. I have observed in a good many instances in which I have failed to detect the amœba in the aspirated liver pus or in the pus which escaped at the time of operation that the parasite appeared, often in great profusion, four or five days later in the discharge from the drainage-tube.’

*Literature.*—The literature of amœbic dysentery is already large, and, besides the authors referred to above, the following should be consulted :

Cunningham : *Quart. Journ. Microsc. Sci.*, vol. xxi., p. 234, 1881.

Councilman and Lafleur : *Johns Hopkins Hospital Reports*, vol. ii., p. 395, 1891.

The remainder of the literature up to 1898 is given by Robert Behla in his small monograph, ‘Die Amœben, vom parasitären und culturellen Standpunkt.’ Hirschwald : Berlin, 1898.

D. G. Marshall, *Brit. Med. Journ.*, June 10, 1899.

### *The Study of Amœbæ, etc., in the Living State.*

The difficulty of obtaining any one kind of protozoa alive without the presence of other protozoa or of bacteria is almost insuperable, and in the case of amœbæ this is easily understood when we reflect that living bacteria, etc., form their staple diet.

Celli and Fiocca\* have made some very successful experiments in the artificial cultivation of amœbæ. They found that a 5 per cent. agar-jelly, made with or without

\* Celli and Fiocca, *Cent. für Bakt.*, April 7, 1894.

bouillon, and strongly alkaline, poured without filtration into Petri-dishes, gave good results. Amœbæ grew and multiplied on this medium, but were always accompanied by growth of bacteria, and careful experiments were made in vain with the view of obtaining amœbæ without bacteria. For hanging-drop preparations the agar was filtered, and rendered alkaline with potassium hydrate or sodium carbonate solution. By picking out amœbæ with the platinum loop the life-cycle of different amœbæ could be studied in the hanging-drop under the microscope.

It would appear that thus far the only way of cultivating amœbæ is to grow them in a culture of bacteria or yeast-fungi, just as certain as the mycetozoa can be grown in similar circumstances.\*

Beyerinck† has thus cultivated an amœba obtained from earth in a growth of nitrifying bacteria on a basis of agar-jelly from which the organic salts had been removed by repeated washing, and a solution of ammonium-phosphate added.

The first difficulty in attempting to obtain bacteriologically pure cultures of protozoa is in their isolation. The visual method—*i.e.*, securing an isolated protozoon by means of the platinum loop under the microscope—as used by Celli and Fiocca, has been also employed by Fünk. It is neither sure nor delicate enough to satisfy anyone who is familiar with bacteriological methods. Another visual plan open to the same objection is isolation by the aid of capillary tubes. Ogata‡ used tubes 10 to 20 centimetres long and 0·4 to 0·6 millimetre in diameter.

\* T. Chrzaszez, *Cent. für Bakt.*, April 4, 1902.

† Beyerinck, *Cent. für Bakt.*, February, 1896.

‡ Ogata, *Cent. für Bakt.*, 1893, No. 6, p. 125.

A sterile nutrient liquid is made and the capillary tube is nearly filled with it; into the empty portion of the tube (1 to 2 centimetres) a liquid containing the living protozoa and bacteria is allowed to flow. Owing to their more rapid movements, the protozoa pass into the sterile part of the liquid in the tube, and they may thus travel for 2 or 3 centimetres or more in the space of five to thirty minutes, whilst the bacteria did not travel so far. When some of the protozoa had thus travelled some centimetres from the place of union of the two fluids, a mark is made on the tube under the microscope, and the tube is broken at a point free from bacteria. The ends of the tube are then sealed. In this way Ogata isolated several of the infusoria; of these, *Polytoma uvella* and *Paramæcium aurclia* were found to multiply on nutrient gelatin, forming in the course of two or three weeks small white colonies, without liquefying the nutrient medium. Flattened capillary tubes have been used by Danielewsky for isolating protozoa.

C. O. Miller,\* using different infusions (such as hay infusion with  $\frac{1}{2}$  per cent. grape- and  $\frac{1}{3}$  per cent. milk-sugar), was able to make twenty-five cultures of amœbæ, but he regarded the presence of living bacteria as necessary.†

\* C. O. Miller, *Cent. für Bakt.*, Bd. xvi., 1894, No. 7.

† For further observations on the cultivation of protozoa see Chapter X.

## CHAPTER III

### THE SPOROZOA

THIS class of the protozoa are all parasitic. With but one or two doubtful exceptions they are all cell-parasites: that is to say, every individual sporozoon that arrives at maturity must have passed a definite period of its life-cycle within one of the cells of some other organism. These parasites are very widely distributed in the animal kingdom. Apart from the protozoa and the cœlenterata (jelly-fishes, sea-anemones, and corals), they have been met with in all classes of animals. Not only are all sporozoa parasites, but they are necessary parasites. No new generation can complete its cycle of existence without invading the cells, tissues, or cavities of an appropriate host. Thus the sporozoa cannot be 'cultivated' in the bacteriological meaning of the term. The dependence of the parasite on the host in the case of the sporozoa is of the closest character. The parasites of malaria require an interchange of hosts similar to that we are familiar with in the tapeworms.

*The seat of parasitism* on the part of the sporozoa varies in different cases. Some species are polyphagous: they can invade and inhabit almost any of the tissues or organs of their host; thus the parasite (*Glugea bombycis*) that

causes disease in silk-worms invades all the tissues of its host, and that at all stages of development, from egg to moth. One species (*Coccidium salamandræ*) invades the nuclei of the epithelial cells of the intestine of the salamander.

*Nutrition* in the sporozoa is effected solely by absorption of fluids. This absorption probably takes place from the surface of the parasite during its period of growth. Other general features of the class will best be gathered from a brief study of typical examples of the chief divisions of the sporozoa. Perhaps, since the parasites of malaria are to medical men now the most familiar of the sporozoa, it will be more profitable to give a brief sketch of them before passing to consider the remainder in their biological order.

#### HUMAN MALARIA.

Three different species of sporozoa are known to produce as many forms of malaria in man. They are :

1. *Plasmodium*\* *malaria*, the parasite of quartan ague, which completes its asexual life-cycle in seventy-two hours.

2. *Plasmodium vivax*, the parasite of tertian ague, which completes its asexual cycle in forty-eight hours.

3. *Plasmodium præcox*, the parasite of quotidian or pernicious malaria. The time required by this species to

\* Many suggestions have been made to improve the terminology of the malaria parasites. They are usually known by the above designation, but they are certainly never plasmodia; hæmamœba is another name equally inappropriate, since these parasites are sporozoa. *Hemosporidium febris quartanæ, tertianæ, perniciosæ* respectively, would, it appears to me, be better.

complete its asexual cycle is not yet quite settled. Celli\* gives it at forty-eight hours.

*Plasmodium Malariae*.—In its earliest intracorporeal stage the young parasite of quartan ague appears as an unpigmented droplet, of easily distinguishable contour, in the interior of a red blood corpuscle, 1. As it grows, 2, 3, it puts out pseudopodia, and rather coarse grains or bars of black pigment appear within it. The movements of the pseudopodia and the streaming motion of the proto-

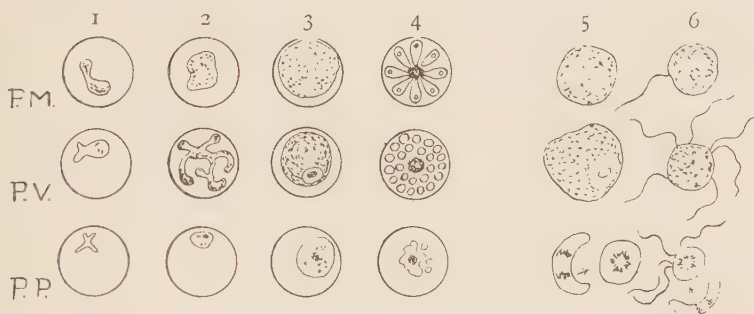


FIG. 27.—DIAGRAM SHOWING THE THREE DIFFERENT SPOROZOA THAT CAUSE MALARIAL FEVER IN MAN.

P.M., *Plasmodium malariae* (quartan parasite); P.V., *P. vivax* (tertian parasite); P.P., *P. praecox* (the parasite of quotidian or pernicious ague). In each case 1 to 4 represent the asexual cycle, and 5 and 6 represent respectively the female and male elements (macro- and micro-gametocytes, etc.) of the sexual cycle. (Abbreviated from Golgi and others.)

plasm, as indicated by that of the pigment, are sluggish. The parasite grows till it almost fills the blood corpuscle, but the latter is neither enlarged nor discoloured. As the parasite reaches maturity—*i.e.*, towards the end of the apyrexial period—the pigment tends to collect in the middle of those of the parasites that are destined presently to undergo asexual subdivision (schizogony; Fig. 27, 4).

\* Celli, 'Malaria,' translated by Eyre, 1900.

The adult sexual forms (gametocytes) are recognisable by the streaming movement of their protoplasm, as evidenced by that of the pigment granules. Having attained a certain size, the gametocytes escape from the corpuscles, and still farther increase in size. The details of fertilization, etc., have not been followed so closely as in the other forms. In preparations stained with methylene blue and eosin\* the youngest quartan parasites show as small blue rings with one or two red nuclear dots. This has been termed the signet-ring stage. It occurs also in *Plasmodium præcox* (see Fig. 27, 2).



FIG. 28.—*PLASMODIUM MALARIÆ* (SCHIZOGONY) AS SEEN IN STAINED PREPARATIONS. (After Bastianelli and Bignami, from Lühe.)

*a, b*, Stages of growth; *c, d*, nuclear division; *e*, separation of the young brood, and disintegration of the host-corpuscle.

*Plasmodium Vivax*.—The parasite of tertian ague, in its early intracorpuscular stage, exhibits a lively amœboid movement; the pseudopodia are seen to be rapidly protruded and retracted.

The pigment consists of fine granules, and by their movement they betray a rapid streaming in the protoplasm of the parasite.

The action of the parasite causes a loss of colouring matter in the red blood cell in which it lives. As the adult forms of *P. vivax* are so large ( $8-10\ \mu$ ) that they enlarge

\* For methods of preparation see Chapter X.

the corpuscle, the pallor of the corpuscle may make it difficult to distinguish between a free and an intra-corpuscular *P. vivax*. After escaping from a host-cell, *P. vivax* may attain to two or three times the size of a red blood cell. This species of parasite causes another change in the infected corpuscles, which becomes evident in specimens stained in certain ways.\* The substance of the corpuscle undergoes a granular degeneration, the granules staining† in the same way as the nucleoli of the parasite (Fig. 29).

In asexual multiplication the pigment lies for the most

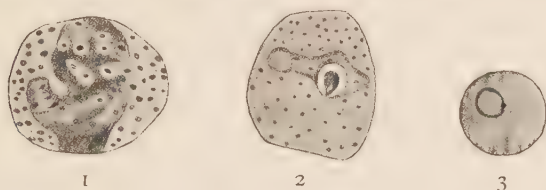


FIG. 29.—STAINED PREPARATIONS OF *P. VIVAX* AND *P. PRÆCOX*.

1 and 2 shows the granular change caused by *Plasmodium vivax* in the red blood corpuscles (after Maurer); 3, ring-form of *P. præcox* (after Mannaberg, from Doflein).

part clumped together near the middle of the parasite, and numerous offspring (usually fifteen to twenty) are irregularly grouped outside this (Fig. 27).

*Plasmodium Præcox*.—The parasite that determines the gravest form of malaria is smaller than the two other species; as a rule, the largest do not occupy more than one-fourth of the volume, or about two-thirds the diameter, of the corpuscle. It is most readily diagnosed by establishing the presence of the crescent form, which only occurs

\* *E.g.*, by hæmatoxylin and by Romanowsky's method; see Chapter X.

† G. Maurer, 'Die Tüpfelung der Wirtzelle des Tertiana Parasiten,' *Cent. für Bakt.*, vol. xxviii., 4-5.

in *P. præcox*, and is therefore diagnostic. The pigment consists at first of very fine, almost invisible, granules; as the parasites grow it collects centrally in the crescents around the nucleus. The young parasite, both before and after it enters into its host-corpuscle, appears, like the quartan parasite, to contain a vacuole, since in stained preparations it usually has the same signet-ring form (Fig. 29, 3). It exhibits a lively amœboid movement, and changes its position in the blood-corpuscle, disappearing and reappearing according as it is in or out of focus. The asexual subdivision results in from seven



FIG. 30.—*P. PRÆCOX*. (After Mannaberg, from Doflein.)

A-C, Schizogony of *Plasmodium præcox*; D, section of a capillary of the brain blocked by *P. præcox*. (From stained preparations.)

to sixteen young parasites of from 1 to 1.5  $\mu$  in diameter. The period occupied in the completion of the asexual cycle in *P. præcox* is more difficult to follow than in the case of the two other forms, because the subdivision occurs chiefly in the bones and the spleen, and parasites in the act of dividing (Fig. 30) are difficult to find. In cases that have proved fatal, with coma preceding death, however, when the capillaries of the brain are examined soon after death, they are found to be blocked with parasites, many of which are undergoing fission (Fig. 30, D). Celli puts the time occupied by the asexual cycle at forty-eight hours,

but the generations overlap, so that the fever tends to be continuous.

The young sexual forms of *P. præcox* have long been known as the 'crescents' of Laveran; they are diagnostic of pernicious fever. They occur in the circulating blood and abundantly in the bone marrow, where they may be found side by side with the asexual forms. They are characterized not only by their form, but by the high power of refracting light that they possess.

In the alimentary tract of the *Anopheles* mosquitoes such of the crescents as are male cells (microgametocytes) become rounded in form; their nuclei divide into from four to seven daughter nuclei, which pass to the periphery of the parasite, and there form the flagellulæ or microgametes, which are the equivalents of the spermatozoa of the higher animals. The female crescents, or macrogametocytes, also become rounded and escape from the blood corpuscles, and become 'mature' by the extrusion of part of their nucleus in a manner similar to the formation of the 'polar bodies' in the ovum cells of the metazoa, and then fertilization is effected by the passing of a single flagelliform microgamete into the substance of the mature macrogamete. This step was first actually observed by MacCallum\* in malaria of birds. Ross,† having first obtained some evidence that the crescents of human malaria underwent farther development in the stomach of gnats, first fully traced‡ the

\* W. G. MacCallum, 'On the Hæmatozoon Infection of Birds,' *Journ. Expt. Med.*, Baltimore, vol. iii., 1898.

† Ronald Ross, 'On Some Peculiar Pigmented Cells found in Two Mosquitoes fed on Malarial Blood,' *Brit. Med. Journ.*, 1897.

‡ Ronald Ross, 'The Rôle of the Mosquito in the Evolution of Malarial Parasites,' *Lancet*, 1898; and 'Life-History of the Parasites of Malaria,' *Nature*, 1899, etc.

sexual cycle of the parasites of avian malaria in gnats of the order *Culex* (Fig. 31, *a*).

The salient points of difference in the appearance of gnats of the two genera as they appear when at rest is shown in Fig. 31, and the appearance of the larvæ in water in Fig. 32.

Grassi has confirmed Ross's observations in the genus



FIG. 31.—THE RESTING POSITION OF *CULEX* (*a*) AND *ANOPHELES* (*b*). (After Waterhouse.)

*Anopheles* infected by human malaria. Several species of *Anopheles* have been proved to be the intermediate hosts for the parasites of all three kinds of human malaria; but since the crescents or sexual cells are most readily followed, the details are most completely known with



FIG. 32.—THE POSITION OF THE LARVÆ OF *CULEX* (*a*) AND *ANOPHELES* (*b*) IN WATER. (After Nepveu.)

regard to *P. præcox*, and the sketch of the life-history of that parasite may now be resumed and be taken to apply to the other two forms.

The alimentary tract of the gnat or mosquito is represented diagrammatically in Fig. 33.\* The microgametes.

\* For further details of the sucking apparatus see L. W. Sambon, 'Anopheles Maculipennis,' *Brit. Med. Journ.*, January 26, 1901.

which are sometimes seen under the microscope in blood drawn from the human subject, and after it has undergone cooling under the cover-glass, are formed most abundantly in the alimentary tract of the gnat during the first two days after it has fed upon an infected person. The

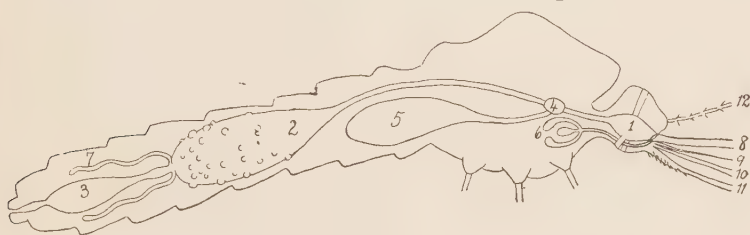


FIG. 33.—DIAGRAM OF THE INTESTINAL TRACT, ETC., OF CULEX.  
(Combined after Ross and Nepeveu.)

1, Pharynx; 2, chyle stomach (studded with oocysts); 3, intestine with ampulla; 4 and 5, suction stomachs; 6, salivary gland; 7, Malpighian tubes; 8, sheath of proboscis, labrum or upper lip; 9, hypopharynx; 10, mandibles and maxillæ (paired); 11, labium or lower lip; 12, antennæ (paired).

fertilized macrogamete of the malaria parasites of man and birds does not, as do other coccidia, at once secrete a thick capsule, but it changes to a spindle shape about  $20\mu$  in length, and at the same time becomes motile, and in this



FIG. 34.—THE OOKINET OF PLASMODIUM PRÆCOX.  
(After Grassi, from Lühe.)

stage is termed an ookinet, or vermiculus (Fig. 34). About forty-eight hours after the blood has been taken into the 'stomach' (midgut) of the Anopheles, the ookinets of *P. præcox* can be seen in worm-like movement. They soon enter into one of the epithelial cells lining the gnat's

bowel, and after resting there a short time they pass deeper, to lie between the epithelium and the elastico-muscular layer on which it rests. Here the ookinet ceases its movement and becomes an oocyst, which is at first of an oval shape, but, increasing greatly in size (from 30 to 70  $\mu$ ), it finally becomes spherical. According to Grassi, the oocyst does not form its own capsule, but this is formed by the elastico-muscular layer of its host. The full-grown oocyst bulges on a short peduncle into the body-cavity of the gnat, pushing before it the layer of fatty cells which cover the elastico-muscular membrane. These large cysts contain pigment, and are found almost exclusively in the dilated part of the midgut, the so-called stomach of the gnat (Fig. 33, 2).

During the growth of the oocyst the nuclei increase in number, apparently by amitotic subdivision. In the fresh state these nuclei resemble vacuoles and refractile granules. Ross first observed the formation of elongated radiating filaments on the fourth or sixth day after the gnats had been fed. These filaments are the sporozoites. In these parasites and in other hæmosporidia there appears to be no sporocyst formed; the foci (representing the sporoblasts of other coccidia) from which the sporozoites are developed are, according to Celli, connected to one another by protoplasmic bridges. The number of sporozoites that may be formed from one oocyst is very great; Grassi has estimated it at 10,000. Each sporozoite has a nucleus derived by repeated subdivision from the originally single nucleus of the fertilized macrogamete or copula.

The sporozoites are arranged about residual segments of the protoplasm of the oocyst. They are at first round,

but become elongated, their final dimensions being  $14 \times 1 \mu$ ; their protoplasm is dense, homogeneous, and highly refracting. The sporozoites developed in the same oocyst reach maturity at the same time. When the formation of the sporozoites is completed the oocyst



FIG. 35.—DIAGRAM REPRESENTING THE LIFE-CYCLE OF THE PARASITES OF MALARIA.

1, Sporozoite; 2-4, asexual cycle; 5, 6, microgametocyte, macrogametocyte; 7, separation of microgametes; 8, maturation of macrogamete; 9, copula; 10, ookinet; 11, part of the gnat's intestine and an oocyst with nuclear division; 12, oocyst with liberation of sporozoites; 13, part of the gnat's salivary gland with the ducts, sporozoites in the cells and in the lumen.

ruptures into the body-cavity of the gnat. The sporozoites, like the microgametes and ookinets, have a snake-like mobility, and in the fluid of the body-cavity they wander to all parts of the gnat's body; but by some attraction they finally collect in the salivary glands, penetrating the cells

and entering the lumen of the canal with the secretion. When the gnat thus charged sucks the blood of a human being the sporozoits are forced into his blood and the whole cycle begins anew. This sexual cycle (cycle of Ross) requires about eight days, and the most favourable temperature is from  $28^{\circ}$  to  $30^{\circ}$  C. At temperatures below  $18^{\circ}$  C. the formation of sporozoits fails. It will now be profitable to rehearse this complicated life-history by means of a diagram (Fig. 35). A separate account of the parasites of avian malaria will be given below (p. 84) in its proper place among the other sporozoa.

The capacity of the infected *Anopheles* to transfer the malarial parasites to man has been made certain by searching experiments by Grassi and Bignami, Manson and the late P. Thurburn Manson. The last-named made himself the subject of an inoculation experiment, and his own account of the result may be quoted\* as at once an example of the clinical features of a mild attack of ague and of the experimental proof of the power of gnats fed in Rome on the blood of a person suffering from benign tertian fever, and sent from Rome to England in gauze cages, to convey the infection to a previously healthy man :

‘The first consignment of mosquitoes arrived at the London School of Tropical Medicine on July 5. Only some half-dozen had survived the journey. They were in a languid condition, and would not feed satisfactorily. One may have bitten me. By July 7 they were all dead.

‘The second consignment arrived on August 29. They had been infected in Rome on August 17, 20, and 23 by being fed upon a patient with a double benign tertian

\* Patrick Manson, *Brit. Med. Journ.*, September 29, 1900.

infection. The patient was reported to have had numerous parasites, including many gametes, in his blood. On arrival twelve insects were lively and healthy-looking. I fed five of them on August 29, three on August 31, one on September 2, and one on September 4. They bit my fingers and hands readily. The bites were followed by a considerable amount of irritation, which persisted for two days.

‘The third consignment arrived on September 10. They had been fed in Rome on September 6 and 7 on a patient suffering from a simple tertian infection, but with very few parasites in his blood. There were some fifty to sixty mosquitoes in good condition. Twenty-five bit me on September 10 and ten on September 12.

‘Up till September 13 I had been perfectly well. On the morning of the 13th I rose feeling languid and out of sorts, with a temperature of 99° F. By mid-day I was feeling chilly and inclined to yawn. At 4.30 p.m. I went to bed with severe headache, sensation of chilliness, lassitude, pains in the back and bones, and a temperature of 101.4° F. Repeated examinations failed to discover any malarial parasites in my blood.

‘*September 14.*—I slept fairly well, but woke at 3 a.m. with slight sweating and a temperature of 101° F. During the day my temperature ranged between 101° and 102° F. The symptoms of September 13 were exaggerated, and anorexia was complete. Several examinations of the blood were made, again with negative result. To relieve headache 10 grains of phenacetin were given at 6 p.m. I perspired profusely, but slept indifferently.

‘*September 15.*—Woke at 7 a.m. feeling distinctly better, with a temperature of 100.4° F. No malaria parasites were

discovered on repeated examinations of my blood by my father. About 2 p.m. I commenced to feel slightly chilly. This soon wore off, and I became hot and restless. By 4.30 p.m. temperature was  $103.6^{\circ}$  F. It remained about  $103^{\circ}$  till 9 p.m., when profuse sweating set in. I am told there was some delirium.

*'September 16.*—I woke at 8 a.m. feeling quite well; temperature  $98.4^{\circ}$ . I made several blood examinations, and found one doubtful half-grown tertian parasite. In the afternoon and evening there was a recurrence of fever (temperature  $102.8^{\circ}$ ), relieved by sweating.

*'September 17.*—Again felt quite well on waking after a good night's sleep; temperature  $99^{\circ}$ . At 10 a.m. several half-grown parasites, a gamete, and two pigmented leucocytes were discovered in the first blood-film examined. During the day many tertian parasites were found. Their presence was verified by my father and others. About 2 p.m. the sensation of chilliness returned; temperature  $101.8^{\circ}$ . By 5 p.m. temperature had reached  $103^{\circ}$ . There was then copious sweating. The edge of the spleen could be felt on deep inspiration, and there was a slight feeling of discomfort in the region of that organ. Dr. Frederick Taylor and Mr. Watson Cheyne confirmed the presence of splenic enlargement. By 9 p.m. the temperature had fallen to  $99.2^{\circ}$ , and I was feeling better. Quinine (10 grains) was given.

*'September 18.*—Woke after a good night feeling perfectly well; temperature  $97^{\circ}$ . Ten grains of quinine were taken, and subsequently 5 grains every eight hours. I continued perfectly well all day. A few three-quarter grown tertian parasites and some gametes were found during the forenoon and afternoon; they were seen by

Dr. Oswald Browne, my father, and myself. At 10 p.m. the parasites had disappeared, the last being found at 5 p.m.

'September 19.—No parasites discovered. Temperature normal. Feeling quite well. There is no splenic enlargement and no tenderness. Appetite returned.

'September 25.—In good health. No recurrence of malarial symptoms.'

After an interval of nine months Manson\* had a recurrence of malaria, after temporarily leaving London for Aberdeen.

It is notable at what long intervals recurrence may occur. It has been my privilege to hear Patrick Manson, at the Seaman's Hospital, urge an abstemious life on malarial patients about to be discharged as cured, and add this warning: 'You may forget your fever, but your fever will not forget you.'

\* P. T. Manson, *Brit. Med. Journ.*, July 13, 1901.

## CHAPTER IV

### GREGARINIDA

THE gregarines were till recently the best known of the sporozoa. They have a very wide distribution among the cold-blooded animals; hitherto none has been found as a parasite in a warm-blooded animal.\*

Those who wish to familiarize themselves with the general characters of the sporozoa and with the methods of examining these interesting parasites cannot omit some study of this order of the sporozoa. The higher gregarines, in their mature state, present two or three divisions of the cell: the posterior, or *deutomerit*, contains the nucleus; in front of this is the *protomerit*; and the *epimerit* again anterior to the protomerit. An example of this group of gregarines is to be found in the common

\* On May 2, 1893, I showed before the Pathological Society of London a section containing gregarines in various stages. This section at the time I thought came from a tumour of a cat's lip. Subsequent investigation has shown me that the section is really one of an earth-worm's seminal vesicle. It was handed me among a number cut from the tumour referred to by an assistant, who had evidently not cleaned the razor, which had been previously used for making sections of an earthworm's seminal vesicle. It is interesting that, as reported in the *Brit. Med. Journ.*, May 6, 1893, p. 951, some experienced pathologists who examined these pseudonavicellæ of the gregarines of the earthworm denied that they were spores or had to do with sporozoa; so little attention had pathologists at that time given to the sporozoa.

brown centipede (*Lithobius forficatus*, Fig. 36). The alimentary canal of this arthropod contains slender bodies about  $\frac{1}{2}$  millimetre in length, of a whitish colour by reflected light. They can be seen by the naked eye, and by the aid of needles one can be easily isolated and placed under a low power of the microscope. The parasite has the appearance shown in Fig. 37. It has a distinct cuticle, which is thrown out into a number of hook-like



FIG. 36.—A COMMON CENTIPEDE, *LITHOBIUS FORFICATUS*.  
(Natural size.)

processes in the anterior segment of the parasite, the epimerit (Fig. 37, *ep*). These cell-organs serve the purpose of enabling the parasite to keep its hold on the host-cell for a longer time than would otherwise be possible. The epimerit separates from the rest of the parasite when full maturity is attained. The body of the young parasite has a dense appearance.

When fixed, stained, and cleared, among other features, a prominent nucleus is brought into view. In the encysted

couples the distinction between protomerit and deutomerit disappears.

In another common brown centipede (Fig. 38), usually found where lithobius abounds, and which has twenty-two pairs of appendages, including the mandibles, another gregarine, nearly 1 millimetre in length (Fig. 39), is found; its epimerit is provided with blunt processes.

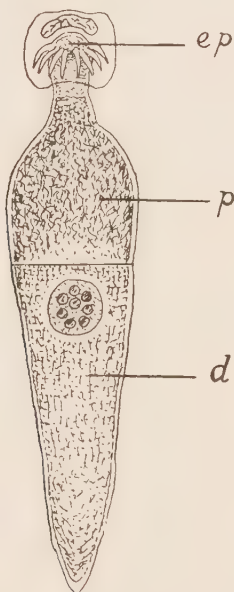


FIG. 37 (*bis*).—A GREGARINE FROM A COMMON CENTIPEDE, LITHOBIUS FORFICATUS.

There is no division of the main part of the body of the animal into proto- and deuto-merit.

In a third kind of centipede, that occurs near Ewhurst in Surrey, and resembles the brown lithobius (Fig. 36), save that the body is of a paler colour generally, and the limbs are marked by alternately darker bands, and the mandibles are longer and more like the locomotor appendages, there

occurs a more rounded gregarine (Fig. 40), which also is readily visible to the naked eye as a white spheroidal body.

These examples serve to show the variety in form presented by gregarines occurring in nearly allied hosts. In all three examples the adult forms are easily

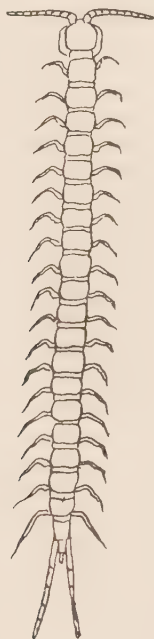


FIG. 38.—ANOTHER COMMON CENTIPEDE.

(Twice natural size.)

recognised by the naked eye, and are readily manipulated by needles. Side by side with the gregarines in the two first-named centipedes, a coccidium, *Adelea ovata*, is often present, and in the intestine of lithobius another coccidium (*C. Schubergi*) is sometimes present in enormous numbers. Most gregarines inhabit the surfaces of hollow viscera, but

one occurs in the body cavity of the larva of *Tipula*, the common 'daddy long-legs.' And although no gregarine is known to cause a definite disease, yet they often occur in enormous numbers, and then they must be a tax on the physiological powers of their hosts.

*Structure.*—In the larger gregarines the ectoplasm often has a complicated structure, presenting first a cuticle, which is longitudinally furrowed, and which provides hooks and similar appendages when these are present; second, a



FIG. 39.—GREGARINE FROM THE CENTIPEDE SHOWN IN FIG. 38.  
× 40.

slime-secreting layer, which confers on the animal the power of progression in a straight line that is so frequently observed when gregarines are examined in their natural state under the microscope. The furrows of the surface direct the slimy secretion backwards. The secretion, by adhering to the surface on which the gregarine rests, and becoming hard immediately after it is formed, pushes the animal forwards. A similar means of progression would explain the movements of other sporozoa,

such as the sporozoits of malaria. Beneath this slime layer is the true ectoplasm, a thin layer of dense protoplasm; it is seen as the partition between proto- and deuto-merit in the septate gregarines. Beneath the ectoplasm a layer of transverse muscle-like fibrils can sometimes be discerned; it is by virtue of these fibrils that some of the gregarines can contract their bodies into crescent form (see below Fig. 45). The *endoplasm* as a rule is dense and filled with coarse granules, most of which consist of paraglycogen, and which obscure the clear, structureless ground-substance, save in young individuals.



FIG. 40.—A GREGARINE FROM A PARTI-COLOURED CENTIPEDE.

Besides the paraglycogen granules, carminophile granules, fat-drops, etc., are met with in various species. A yellow, brown, or red colour is possessed by some gregarines. The above properties are worth mention for the purpose of comparison with other sporozoa; but it is not proposed here to deal in any detail with the gregarines, a few examples of the more readily accessible varieties may, however, be given.

#### *Clepsidrina Blattarum.*

The alimentary canal of the cockroach, which is in my experience constantly infested by this gregarine, is easily

exposed by dissection. After decapitation an animal is fixed by pins and shorn of its wings and terga. The body cavity is then seen to contain in front the large crop, followed in turn by the conical gizzard, the midgut, with seven or eight yellow tubes (hepatic cæca) entering its anterior, and many slender nephridia its posterior end, the short ileum, the wider, coiled colon, and finally the rectum, marked by longitudinal grooves. For histological investigation the midgut is the most interesting part of the intestine, and can easily be distinguished, separated by scissors, and put into hardening fluids. The small valve



FIG. 41. — LONGITUDINAL SECTION THROUGH PART OF THE ALIMENTARY TRACT OF A COCKROACH. (Enlarged about 4 diameters.)

1, Gizzard, prolonged into the valve; 2, Midgut, with the hepatic cæca to the left, the nephridia to the right; 3, colon.

between the gizzard and the midgut will lie in the anterior portion, and the recess between its outer wall and the epithelium lining the midgut is a favourite seat of the parasites.

When highly magnified the epithelium appears to be ciliated as in Fig. 42, copied from one of Nussbaum's drawings which illustrate Max Wolters'\* paper on conjugation and spore-formation in gregarinæ. Some of the intracellular parasites here shown resemble certain cell-inclusions in cancer.

The parasites sometimes attain large dimensions, and

\* Max Wolters, *Archiv für Mikroskop. Anat.*, vol. xxxvii.

become reticulated before leaving the cell. Bütschli represents them on the point of becoming free, with the epimerit still embedded in the host-cell. But sections (see Fig. 43) show plainly enough that many become free before reticulation occurs, and while they still possess a



FIG. 42.—FOUR EPITHELIAL CELLS FROM THE INTESTINE OF A COCKROACH. (After Nussbaum.)

The nuclei are pale, with a few points of chromatin. The two central cells contain each two parasites; in the second cell from the left the parasite above the nucleus has a small protomerit, which appears as if separated from the larger deutomerit. (Zeiss E, oc. 4.)

protoplasm of the same dense character as those in the cells of Figs. 42 and 43, but are larger. The others are of quite different appearance and of relatively gigantic proportions. These have a highly refracting reticular

protoplasm and a thick cuticle, which, when a parasite is cut transversely, as is seen in the upper of the two to the left of the field in Fig. 43, presents distinct tooth-like projections, due to the longitudinal furrows referred to above. Two of these parasites are cut in complete longitudinal section. In both the protomerit contains granules; in one the nucleus has the character of a flame-nucleus, as described by Max Wolters; the other has a large vesicular nucleus containing numerous spherical nucleoli. The structural details of these fully-developed free forms is brought out equally well with acid hæmatoxylin and with the Ehrlich-Biondi reagent.

The stages which intervene between the small intracellular and the fully-developed phases are given by Bütschli. The young parasites enlarge, become reticulated, and come to protrude from the host-cell, being attached for a time by a small spherical epimerit, which at last becomes free. Bütschli\* observed the conjugation of free parasites in egg-albumin as follows: 'The protomerit of the second parasite becomes attached to the deutomerit of the first, and a rotation of the two together takes place. The two parasites gradually form a more compact mass, at first globular, and come to rest at the same time as a gelatinous envelope is formed around them, and within this a second definitive capsule, which has the oval form finally assumed by the encysted syzygium.' L. Pfeiffer† has in an allied species observed conjugation commence within epithelial cells containing two or more parasites (*Mehrlings-infektion*), and also the encystment of solitary parasites without any previous conjugation, which would

\* Bütschli, *Zeitsch. für Wiss. Zool.*, Bd. 35.

† L. Pfeiffer, 'Protozoen als Krankheitserreger,' p. 6.

thus seem to be unnecessary to the spore-producing process.

After from four to seven days in weak glycerin and water preparations, or in fæces, some of the parasites will be found to have formed spores. These are small barrel-shaped bodies ( $2-4\ \mu$ ) which contain a distinct



FIG. 43.—SECTION THROUGH PART OF THE MIDGUT OF A COCKROACH.

Below epithelium, above free parasites, as described in text. Many of the epithelial cells contain young gregarines. (Zeiss F, oc. 2.)

nucleus. Bütschli describes their formation from the peripheral layer of the parasite and their migration to the central part. The spores are evacuated by special organs, the sporoducts, which first appear as small clear circles surrounded by the reticular remains of the substance of the parasite; afterwards they become everted

and appear as delicate tubes with a thickening at their points of insertion. Bütschli explains the evacuation of the spores by supposing that the protoplasmic reticulum contracts and forces them out.

*The Gregarines of Earthworms.*

The common earthworm provides a universally accessible source of gregarines for study; and though the study of its sporozoa is somewhat difficult on account of the complicated structure of the male generative organs,

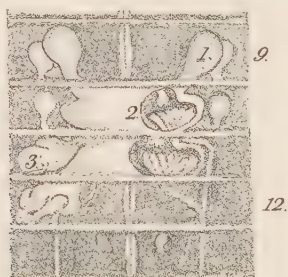


FIG. 44.—THE GENERATIVE ORGANS OF AN EARTHWORM.

1, Anterior seminal vesicle; 2 is placed to the left of the anterior right testis and seminal funnel; 3, one of the lateral seminal vesicles. Segments 9 and 10 contain spermathecae, and segment 13 the ovaries.

and the great number of different parasitic species which are there met with, it is advisable to give some description of the parts constantly infested, because so many points in the biology of these gregarines still remain obscure.

The generative organs lie in the fore-part of the animal, in segments 9 to 14 inclusive. The two pairs of testes in the sexually immature worm, which may be distinguished by the absence of a clitellum, are sometimes to be seen with a lens as bluntly digitate bodies at the back of the septa 9 to 10, 10 to 11. They are solid cellular structures. In

mature worms they are hidden by the seminal vesicles, which unite across the middle line on the ventral side of the œsophagus, coalescing in the tenth, the two posterior pairs in the eleventh, segment. Behind the testes and in the same segment lie the ciliated funnel-shaped ends of the vasa deferentia. The seminal vesicles are the white bodies which are seen on opening the earthworm from the dorsal aspect. They are soft white pear-shaped bodies, which on section are found to contain a cavity subdivided by vascular trabeculæ. The spermatic elements closely simulate sporozoa, and it is necessary clearly to distinguish them. For this purpose I would refer the reader to the paper of J. F. Bloomfield,\* and here only state that the mother cells of the spermatozoa are budded off from the testis as round or slightly moriform cells; as they pass along the seminal funnels the nucleus divides again and again, forming the distinctly moriform spermatospheres, which have a slightly oblique constriction about their middle. In this form they arrive in the seminal vesicles. The rounded projections of the spermatospheres are the 'spermatoblasts.' They multiply by vertical fission to a certain extent, and then put out peripheral points which become elongated into the tail, while the nuclei remain as the heads of the spermatozoa.

Some of these various forms are represented in Fig. 45. The central part of the spermatosphere—viz., that which carries the spermatoblasts—remains unused. This is termed the 'sperm-blastosphere,' and so sometimes the spermatosphere comes to have a superficial resemblance to a gregarina with its brood of sporogonia at its periphery. The parasite, however, has at this stage a distinct capsule

\* J. F. Bloomfield, *Quarterly Journ. Micro. Science*, vol. xx., p. 79.

and minor characters which readily distinguish it. The general arrangement of the male generative organs is given in Fig. 44, which is largely influenced by an illustration in Marshall and Hurst's 'Practical Zoology,' to which work I would refer for farther details. The degree of distension of the seminal vesicles varies. They are fullest just before the physiological generative cycle culminates in the early summer of each year, when copulation occurs.



FIG. 45.—BODIES SEEN AFTER TEASING OUT A SEMINAL VESICLE OF AN EARTHWORM.

Above and to the right two examples of *Monocystis agilis* in different phases of movement. To the left, an encysted syzygium; below, a parasite within a spermatosphere from which project stunted spermatozoa. The remaining four bodies show stages of development of the spermatozoa. (Zeiss D.)

Many varieties of the parasites occur in different individuals of the genera *Lumbricus agricola* and *L. rubellus*. Some of the features of gregarines as commonly seen in sections of an earthworm's seminal vesicle, under a moderately high-power dry lens are shown in the accompanying Fig. 46.

Cuénot,\* who has recently studied the gregarines of the earthworm, describes five different kinds: *Monocystis magna*, which is not found in the seminal vesicles, but in the cup-shaped projection of the vibratile chambers of the male apparatus; *M. lumbrici* (*S. agilis*), in the seminal vesicles; *M. pilosa* (300  $\mu$ ), the cuticle of which is provided with long hair-like processes; and *M. porrecta*, which has a long filiform body.



FIG. 46.—PORTION OF A TRANSVERSE SECTION OF A SEMINAL VESICLE OF AN EARTHWORM.

To the left, part of an encysted sporing parasite with small pseudonavicellæ and reticulate residual substance; below and to the right, an encysted parasite from which sporoblasts have formed; above, part of a cyst containing large sporocysts (pseudonavicellæ).

Recent studies† of the modes of multiplication in gregarines show that a rudimentary sexual process is present in the total conjugation of similar elements, which fuse together to form the sporoblasts.

\* Cuénot, 'Gregarines,' *Archiv. de Biol.*, vol. xvii., 1901.

† E.g., Siedlecki 'Ueb. die geschlechtliche Vermehrung des Monocyst. Ascidie' (Ray Lankester), *Bull. internat de l'Acad. des Sciences*, Cracow, December, 1899.

To recapitulate the life-cycle of gregarines, the following stages may be recognised :



FIG. 47.—DIAGRAM ILLUSTRATING THE LIFE-CYCLE OF A GREGARINE.

- 1, Three epithelial cells showing in turn entry of sporozoite, growth of and escape of parasite; 2, apposition of two gregarines; 3, encystment of two apposed gregarines, with division of 'micro-nuclei'; 4, farther nuclear divisions; 5, formation of sporoblasts; 6, conjugation of sporoblasts; 7 and 8, formation and escape of sporocysts; 9, sporocyst (on larger scale) ruptured, giving issue to eight sporozoites.

1. Period of growth.
2. Association, either early or just previous to sporing.
3. A process of nuclear reduction: the greater part of

each nucleus undergoes degeneration; the remainder, or 'micronucleus,' by successive mitotic divisions, gives rise to a great number of nuclei, which travel to the periphery.

4. Formation of sporoblasts by accumulation of protoplasm around the nuclei.

5. Conjugation of sporoblasts two by two, the nuclei and the cytoplasms fusing completely to form zygotes.

6. Transformation of the zygotes into a sporocyst by the secretion of a sporal membrane.

7. Formation of eight sporozoites in each sporocyst.

The chief features of this cycle are represented in the accompanying diagram (Fig. 47).

### *Ophryocystis.*

This little-known group includes two species, *O. Bütschlii* and *O. Francisi*. They appear to be more nearly akin to the gregarines than to any other group of the sporozoa. In *O. Bütschlii* the full-grown parasites measure 20 to 30  $\mu$ , and have an amœba-like form. The spores measure 12 to 17  $\mu$  by 7 to 8  $\mu$ . The single nucleus multiplies to eight, and as many sporozoites are formed. The cyst is enclosed by many concentric capsules, which split at an equatorial suture. The parasite inhabits the Malpighian tubes of *Blaps mortisaga*.

## CHAPTER V

### COCCIDIA AND HÆMOSPORIDIA

THE whole group of the coccidi-ida has been reconstructed owing to the discovery of the sexual phase of their life-history, and the recognition that the hæmosporidia are closely related to the group. Previously the occurrence of the adenoma-like nodules caused in the liver of the rabbit by *Coccidium oviforme* constituted the chief claim to notice, on account of the basis offered for comparison with human morbid growths; now, whilst still retaining this important claim to notice, they possess other wide biological and pathological points of interest.

For purposes of practical study various genera—*e.g.*, *C. oviforme*, *C. Schubergi*, and the *Caryophagus salamandræ*—may be considered, and the hæmosporidia and pirosuma, on account of their affinity to the malarial parasites and the historical importance of the former.

Sexual phenomena in coccidia have already been touched upon in the consideration of the malarial parasites, and, as has already been mentioned, Macallum actually observed the male enter the female element in the hæmosporidia of birds. The recognition of the sexual characters in the coccidia generally has been a gradual one. Up to the year 1892 the life-history of the rabbit's coccidia was generally thought to be limited to the forms repre-

senting the sexual cycle (Fig. 48). In that year R. Pfeiffer\* showed that there was a dimorphism in these parasites, recognising the asexual subdivision (schizogony). The first recognition of the microgamete phase of *C. oviforme* as an important modification was, I believe, made by myself.† Podwysotszki (in *C. oviforme*), and more particularly Simond (*C. salamandræ*) and Schuberg (in coccidia of mice), farther developed the subject; but to Schaudinn and Siedlecki is due the full demonstration of

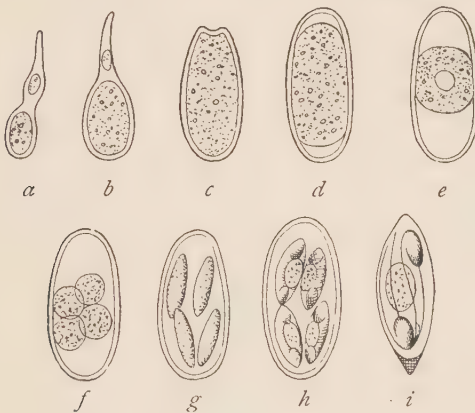


FIG. 48.—COCCIDIUM OVIFORME. (After Balbiani, from Wasielewski.)  
*a, b*, Parasites within the epithelial cells; *c*, encapsulated parasite with flattening of one end of the capsule; *d, e*, shrinking of the protoplasm to a central ball; *f, g, h*, spore-formation; *i*, ripe spore with two sporozoites and a residual body.

the remarkable cell-processes that bring these protozoa closer to the metazoa.

### *Coccidium Schubergi.*

This parasite, the description of which we owe to Schaudinn,‡ is occasionally met with in the alimentary

\* R. Pfeiffer, 'Protozoenforschung,' Berlin, 1892.

† *Brit. Med. Journ.*, 1893, vol. ii., p. 1051. See also Appendix.

‡ Schaudinn, *Zool. Jahrb. Abteil. Anat.*, vol., xiii., 1900.

tract of the common centipede, *Lithobius forficatus*; it is less constant than the gregarine and the coccidium *Adelea ovata*, referred to above. The asexual reproduction may result in infection of nearly every epithelial cell. The latter, as the result of infection, first become swollen,



FIG. 49.—DEVELOPMENTAL CYCLE OF COCCIDIUM SCHUBERGI.  
(After M. Lühe.)

- 1, Sporozoite (or equally well merozoite) entering an epithelial cell; 2, grown schizont; 3, nuclear subdivision; 4, schizogony (asexual multiplication); 5 and 5a, full grown but as yet unripe sexual individuals, macrogamete and microgamete; 6, macrogamete, matured by extrusion of nuclear matter; 6a, microgametocyte, with microgametes in process of formation; 7, fertilization; 8, young oocyst; 9, formation of sporoblasts; 10, formation of spores and sporozoites; 11, sporozoite.

and subsequently undergo fatty degeneration; the parasite absorbs all but a small residual portion of the cell-substance and the nucleus; the grown parasite and cell-remnant fall together into the lumen of the intestine.

Severe infection causes great weakness in the hosts. Recovery is brought about by regeneration of the epithelium, and may be so complete that the host becomes freed from the parasites. Infection occurs from the centipedes preying on other arthropoda, etc., that have eaten earth containing spore-ripe coccidia. The sporocysts appear not to open in the alimentary tract of these intermediary animals, but once arrived in that of lithobius, the sporocysts rupture, liberating the sporozoits. These have dimensions of  $15$  to  $20\ \mu$  by  $4$  to  $6\ \mu$ ; they are slightly sickle-shaped, and are provided with a sharp point at their anterior end. They are motile, not only bending sharply by approximating their extremities, but, like the gregarines (see p. 60), moving forwards by secretion of a gelatinous matter. By virtue of this motility, they bore their way into the epithelial cells (Fig. 49). In twenty-four hours the sporozoits grow to round coccidia; these are devoid of membrane. Their nuclei possess a network of chromatin and a large nucleolus. The nucleus at once begins to undergo binary divisions, and finally the plasma also subdivides, resulting in the asexual formation of a group of nucleated merozoits arranged about a central residual body (Fig. 49, 4). These escape to infect fresh epithelial cells. This asexual spread of infection continues for four or five days; the conditions—sickening of the host, etc.—now necessitate other measures for the continuation of the parasitic life, and on the fifth or sixth day the sexual differentiation of the parasites begins (see Fig. 49, 5 and 5a). After fertilization the oocysts, escaping with the dung, develop spores, and are ready to reinfect other hosts through the intermediary of their prey.

*Coccidium Oviforme.*

The oval oocysts of this parasite were first described by Hake\* as occurring in the rabbit's liver. Leuckart, who applied to it the above term, knew only a part of the life-history of the parasite, the asexual multiplication being unknown to him. The asexual forms vary from 20 to 50  $\mu$  by 20 to 39  $\mu$ , and give birth to from 20 to 200 offspring. The oocysts are slender and oval, measuring from 36 to 49  $\mu$  by 18 to 28  $\mu$  in the liver, those from the intestinal mucous membrane being smaller. The colour of the oocysts is greenish. The cyst-wall is stout, except at one end, where there is a large micropyle. The protoplasmic contents, which at first completely fill the cyst, shrink to a round central mass, which subdivides into four spores, which also become encysted, and in each two sporozoites and a residual body are formed.

From the pathological point of view, it is not sufficient to regard the *C. oviforme* as a tumour-producing parasite. A graduated series of events—from acute to chronic inflammation, thence to tumour-formation and involution—must be taken into account. In wet seasons many young wild rabbits die of acute gastro-enteritis caused by this coccidium.

*C. falciforme*, a genus closely allied to *C. oviforme*, causes a similar disease in mice. In some mice that I watched, the course of the disease was as follows :

'No change was noticed in the animal until six days after the parasites were administered. On this day its coat was noticed to be rough, the abdomen was distended, and the thighs were somewhat drawn up towards the belly, so that the animal's gait was stiff. The stools were

\* Hake, 'A Treatise on Varicose Capillaries,' London, 1839.

softer than normal. The next day blood was noticed about the animal's anus, and this sanguineous discharge contained coccidia. . . .'\*

After death the stomach was found to be distended, the contents consisting almost entirely of coccidia, many of which were subdivided into merozoites. The mouths of the cardiac glands were filled with the latter, some of which had entered the epithelial cells.

The intestines contained little besides parasites, either free in the lumen of the bowel or the glands, or within the epithelial cells of the latter. Many of these presented what is now recognised to be the microgamete stage. I found no tumours in the liver in mice.

*The Red-flux of Cattle.*†—The occurrence of a sanguineous anal discharge in mice infected with coccidia recalls the above-mentioned disease. This disease appears after an incubation period of about three weeks, with high fever and rigors. In the fæces blood appears either in the form of large and small clots or as a blood-stained diarrhœa with diphtheritic membranes. *C. oviforme* is abundantly present in the discharges, and death occurs sometimes within two days in 2 to 4 per cent. of the cases. Recovery occupies from eight to twenty-one days. In fatal cases the mucous membrane of the bowel is reddened, and contains hæmorrhages and diphtheritic membranes. The disease occurs in summer and autumn in the higher Alps, and is due to infection from the soil.

Thus, in rabbits, mice, and cattle coccidia cause endemic disease.

\* J. Jackson Clarke, *Quart. Journ. Microsc. Sci.*, 1895.

† Zschokke, *Schweitzer Archiv für Thierheilkunde*, quoted by Wasielewski, 'Sporozoenkunde,' p. 107.

The coccidial growths in the rabbit's liver, like the lesions in the intestine, show a series of grades of activity. They are conspicuous by their light-yellow colour; the more recent and active, the larger and softer they are. The sites of old and obsolete lesions are recognised as small scars, with a miliary nodule in the centre. A section



FIG. 50.—SECTION OF PART OF THE MARGIN OF A COCCIDIAL LESION OF A RABBIT'S LIVER IN AN EARLY STAGE.

Some of the parasites in this figure are in the early microgamete phase marked by peripheral rods of chromatin.

made through a young, and hence growing, nodule shows great activity, merozoite formation\* and sexual phases being observed side by side (Fig. 50). The same figure shows at the lower part to the right perforation of the

\* The formation of merozoites usually occurs in naked parasites; sometimes, as in Fig. 50, to the left, a delicate capsule is formed. I have described the same feature in the stomach of a mouse (*Quart. Journ. Med. Sci.*, plate 30, fig. 8, 1895).

basement membrane and invasion of the deeper tissues by parasites and epithelial cells. By a similar invasion of the intestinal tissues, in all probability this coccidium (hence also termed *C. perforans*) reaches the radicles of the portal vein, and in this way the presence of the parasites in the liver is to be accounted for.

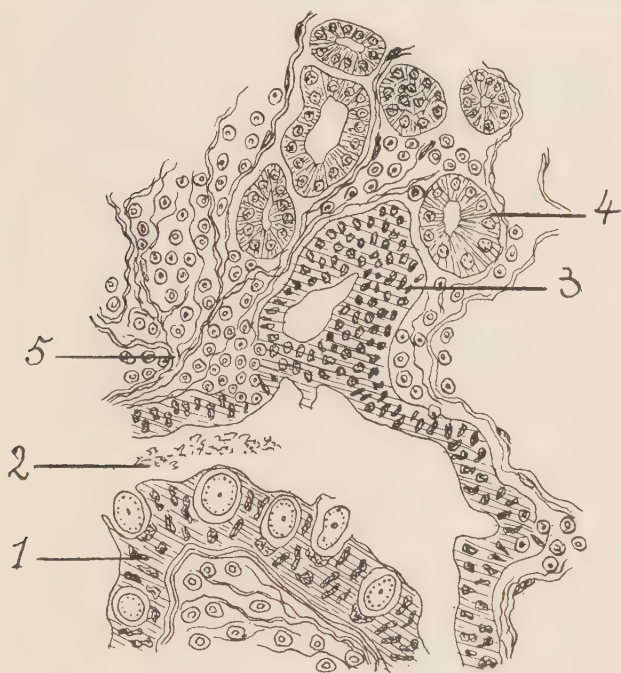


FIG. 51.—SECTION OF A COCCIDIAL LESION OF A RABBIT'S LIVER (LATER STAGE).

- 1, Epithelium containing coccidia ; 2, granular matter in the cavity of the cyst ; 3, proliferating epithelium ; 4, section of a bile-duct ; 5, connective tissue with round-cell infiltration.

A more mature lesion, one that has attained its full growth, shows parasites and pathological processes of less active characters (Fig. 51), the histology of the growth being that of a simple adenoma.

*Coccidium Salamandræ.*

This parasite deserves notice by reason of its property of developing within the nucleus as well as in the cytoplasm of its host-cell (Fig 52). Steinhaus thought that the nucleus was its only habitat, but it was subsequently shown by Drüner that it could also develop in the protoplasm close to the nucleus. The coccidium lives in the intestine of the salamander.

The foregoing coccidia are characterized by the formation of four sporocysts from each fertilized female cell.



FIG. 52.—COCCIDIUM SALAMANDRÆ, FROM THE INTESTINAL EPITHELIUM OF THE SALAMANDER. (After Steinhaus, from Wasielewski.)

*a*, Early infection ; *b* and *c*, formation of asexual brood (merotomy).

The corresponding cells of others of the coccidiomorpha form numerous sporocysts, and are distinguished by the family name Polysporocystidæ. Of this group the genus *Klossia* is the best-known example.

*Klossia Helicina.*

Kloss of Frankfort, in 1855, described the sporozoa which infest the kidney of several species of snail. Schneider first classed the parasite with the Coccidi-ida. I owe my first acquaintance with this parasite to the kind-

ness of Dr. L. Pfeiffer, who sent me from Weimar some infected specimens of *Helix hortensis*. Specimens of the same species of snail that I have found and examined in this country contained the same parasite. As mentioned below, I have found them also in a small variety of gray slugs. They appear to be absent in the black slug, and I have as yet failed to find them in the common snail.

In Fig. 53 the kidney is represented as seen when the mantle of a snail is detached in front and at the sides and



FIG. 53.—A COMMON SNAIL WITH THE SHELL REMOVED AND THE MANTLE DETACHED IN FRONT AND AT THE SIDES, AND TURNED BACK TO SHOW THE KIDNEY, K, WITH THE HEART CLOSE TO ITS LEFT SIDE.

turned back. The heart is seen close to and on the left side of the kidney. In slugs the organ occupies the same relative position as in snails, and can be seen through the mantle and internal shell as a small yellow body.

If the kidney of an infected animal is teased out on a slide with normal salt solution, the larger parasites are easily recognised as oval granular bodies with a large clear nucleus and well-marked nucleolus among the kidney-cells, most of which contain brownish urinary calculi. Some of these parasites are free, others encapsuled

within the host-cell. To the left of Fig. 54 are represented three cells of a snail's kidney seen in vertical section; the central cell and its nucleus are hypertrophied, and the cell contains a large parasite. The lateral cells contain calculi. After a time the nucleus shrinks, and is thrust to one side. In most instances more than one parasite finds access to a cell, but only one comes to maturity; the others remain abortive, and appear as granular masses in the infected cell. The parasite forms a capsule which contains what look like vacuoles, as are

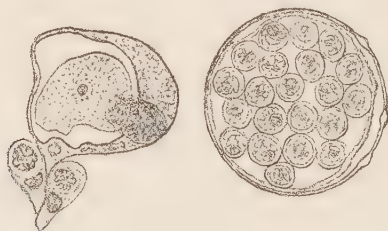


FIG. 54.—*KLOSSIA HELICINA*.

To the left, three cells from a transverse section of the kidney of a snail. The central cell and its nucleus are hypertrophied, and it contains a large parasite. The lateral cells contain calculi. To the right, an encapsulated parasite subdivided into sporocysts, each of which contains four spores and a residual body.

shown to the right of Fig. 54, where also an inner second capsule is present. After repeated nuclear divisions the mass of the encapsulated parasite subdivides into sporogonia. The sporogonia become sporocysts by assuming a capsule, and within each the sickle-shaped spores form, and a round residual body. The sickles are clear, highly refracting bodies, as indicated within the sporocysts to the right of Fig. 54; they can be seen in creeping movement in fresh teasings. They enter the cells, and there growing, some attain the large granular oval form described at the beginning of this section. L. Pfeiffer has

made the important observation that in species of snails with small kidney-cells the parasites attain dimensions proportionally smaller than in the species with larger kidney-cells; and, further, that though the parent parasites are smaller, the sporocysts are of the same size as those of the larger parasites, their number only being reduced.

The sexual processes are somewhat complicated; some of them I first encountered in the kidneys of the small slugs taken from the rocks at the falls of the Shin, in Sutherland.\* They have been more closely studied in another of the Polysporocystidæ, *Adelea ovata*. The asexual individuals are of two sizes, and during a variable period reproduction by schizogony takes place. After this the sexual generation appears: the larger cells subdivide, and these subdivisions, after entering a fresh cell, become the macrogametocytes. By a reduction of nuclear matter they become macrogametes and escape from the host-cell.

The male cells arise directly as the subdivisions of the smaller asexual individuals, and, escaping from the host-cells, they seek the female cells, and, moving to one of the poles of the oval macrogamete, their nucleus undergoes subdivision into four, and as many microgametes are formed. One of these enters the female cell, the others, with the residue of the microgametocyte, adhere to the female cell. After fertilization the nucleus of the latter undergoes repeated subdivision, and an encysted collection of sporocysts (Fig. 54) is formed. In one of the Polysporocystidæ, *Legeria octopiana*, asexual multiplication has not been observed, and the formation of microgametes takes place as in *Coccidium oviforme*.

\* J. Jackson Clarke, *Quart. Journ. Microsc. Anat.*, 1895.

*Nuclear Processes in Coccidia.*

The nuclear changes that occur during the varied life-processes of the sporozoa have been closely studied of late years. The observations of Siedlecki on the nuclear changes in the coccidium of a cuttle-fish\* are especially complete. In this parasite Siedlecki found the asexual cycle was wanting. The nucleus of the adult parasite was as shown in Fig. 55, 1, where for clearness the details of the cytoplasm are omitted. It contains a nucleolus, from which at one part a small knob of chromatin projects, and represents the remains of a bridge of chromatin that at an earlier stage joins the nucleolus to the peripheral chromatin of the nucleus. The maturation of the female cell (Fig. 55, 5) is brought about by budding of the nucleoli; the nucleus approaches the surface of the parasite, and the nuclear matter resulting from the budding is extruded. Other processes are indicated in the description of Fig. 55.

*Hæmosporidia.*

To this subdivision of the Coccidiomorpha belong the parasites of malaria considered above. They are cell-parasites. During their asexual cycle they inhabit the blood-cells of their hosts. The sexual cycle (sporogony) in the better-known instances, human and avian malaria, takes place in an intermediate host. In some of the cold-blooded animals it is not improbable that the whole life of the hæmosporidia—e.g., *Lankesterella ranarum*,† parasitic in the red and white blood-corpuscles, spleen-, liver-, and

\* Siedlecki, *Ann. de l'Inst. Pasteur*, 1898, p. 799.

† Ray Lankester, *Quart. Journ. Microsc. Sci.*, 1871, p. 387.

marrow-cells of *Rana esculenta*—may be passed within the one host.

Of the hæmosporidia of birds the genus *Hæmoproteus* is the better known, and may now be briefly noticed.

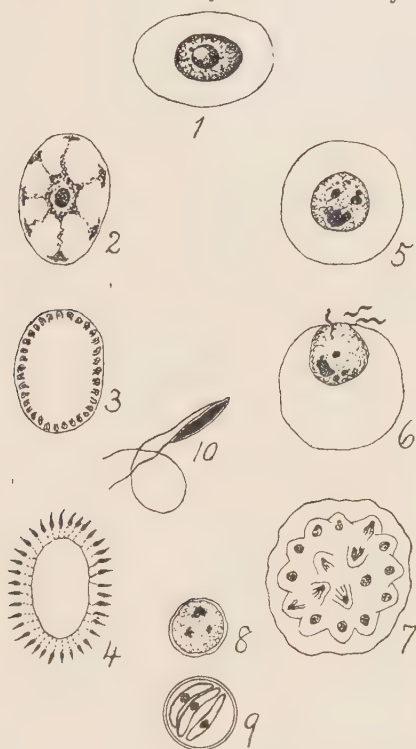


FIG. 55.—SOME NUCLEAR PROCESSES IN COCCIDIA.

- 1, Nucleus of undifferentiated adult coccidium; 2, nuclear matter moving to the periphery preliminary to the formation of microgametes; 3, nuclear matter, having reached the periphery, separates into segments; 4, the microgametes formed; 5, macrogamete; 6, fusion of micro- and macro-gamete; 7, encystment and nuclear subdivision; 8, early stage of sporocyst; 9, sporocyst with three spores (diagrammatized after Siedlecki); 10, microgamete of *Coccidium Lacazei* (after Schaudinn).

*Hæmoproteus Danilewskyi* (Kruse).—The young intracorpuseular forms are of amœboid, irregular form, and free

from pigment. As they grow they become rounder, and pigment is deposited in the cell-plasm which is finely granular. The nucleus is vesicular. Asexual subdivision



FIG. 56.—*HÆMOPROTEUS DANILEWSKYI*. (Doflein.)  
Asexual subdivision with the formation of few schizonts.

is said to occur in two different ways:—either six to seven schizonts are produced in rosette form (Fig. 56) or a

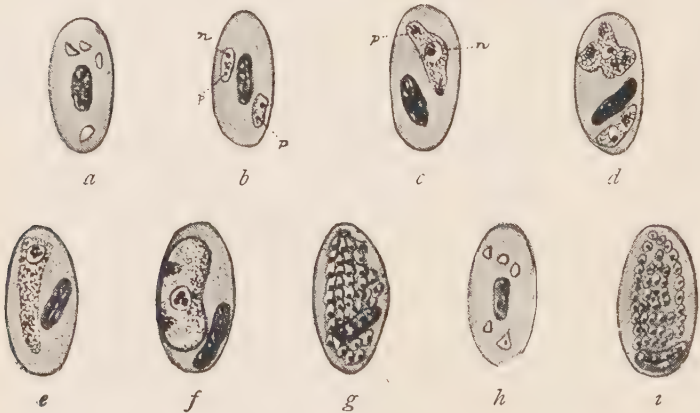


FIG. 57.—*HÆMOPROTEUS DANILEWSKYI*. (After Labbé, from Wasielewski.)

*a-g*, From the blood of a lark; *h* and *i*, from the blood of a finch; *a* and *h*, early multiple infection; *b*, blood-corpuscle containing two parasites (*p*, *p*), the nuclei (*n*, *n*) of the parasites are shown; *c*, blood-corpuscle and an older parasite with nucleus; *d*, blood-corpuscle containing two amoeboid parasites; *e*, elongated parasite which has passed the amoeboid phase; *f*, full-grown parasite shortly before it subdivides; *g* and *i*, numerous brood formed by asexual subdivision (schizogony).

great number almost filling the host-cell (Fig. 55, *g* and *i*), the nucleus of which is often displaced by the parasites.

The gametocytes appear as reniform or pear-shaped bodies. After fecundation has taken place in the intestine of the gnat (*Culex*), the motile cell (ookinet, Fig. 58) penetrates the epithelial coat of the intestine and increases greatly in size. The dilated part of the gnat's intestine, when it contains many of these cysts (Fig. 59), forms a



FIG. 58.—OOKINET OF HÆMOPROTEUS. (After R. Koch, from Doflein.)

striking object, which can readily be detected by teasing out a gnat on a slide and placing it under the low power of a microscope. The formation of sporozoites and their transference from the gnat to birds takes place as in the parasites of human malaria. Many different kinds of

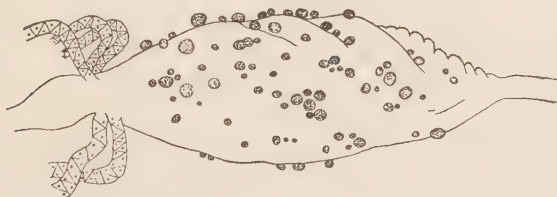


FIG. 59.—PART OF A GNAT'S INTESTINE CONTAINING OOCYSTS OF HÆMOPROTEUS DANILEWSKYI. (After Ross, from Lühe.)

birds are susceptible to the infection: hawks, owls, sparrows and other finches, pigeons, ravens, etc.

Different species of *Culex*, *C. pipiens* in Europe, *C. fatigans* in India, serve as intermediate hosts.

The pathological action on their hosts is similar to that observed in human malaria. The body temperature may

be raised one or two degrees, and the birds become anæmic, mope, and lose their appetite. The distribution of the parasite is a wide one; it occurs in Europe (England, France, Germany, Italy, and Russia), America, Asia, and Africa.

Another hæmatozoon, *Halteridium Danilewskyi*, that infests the blood of larks and other birds, is characterized by a binary division of the whole parasite preliminary to schizogony.

*Pirosoma Bigeminum.*

This parasite, the life-history and affinities of which are only partially worked out, is the cause of a disease of cattle known and feared in the United States as 'Texas fever'; it has also been named 'hæmoglobinuria of cattle' from a leading symptom. The disease occurs also in the north of South America. The parasite, like the hæmosporidia, passes one stage of its life-cycle within the red blood-corpuscles of its hosts. The commonest form in which it occurs is as a pear-shaped body (Fig. 60, *h, j, k*). Frequently these bodies occur in pairs.

Smith and Kilborne,\* who discovered the parasites, describe the earliest stage as minute corpuscles  $0.5\ \mu$  (Fig. 60, *a*) in diameter. They become biscuit-shaped (Fig. 60, *b, c, d*). Doflein, in a preparation of spleen-blood, found clusters of three or four subdivisions; this stage appears to correspond to schizogony in the hæmosporidia. The other larger pear-shaped parasites appear, from the observations of Lignières, probably to correspond to the sexual stage of hæmosporidia. When watched in fresh

\* Smith and Kilborne, *Bull. Dep. Agric. Am. Industr.*, No. 1, p. 67, 1893, quoted by Doflein, *loc. cit.*, p. 150.

blood-preparations under the microscope they change their form, becoming spherical. There is some evidence that microgametes are formed as in the hæmosporidia. Another point of similarity to the better-known hæmosporidia is the fact that an intermediary host appears to be required by the parasites for the conveyance of infection. This intermediate host is a tick, the *Boophilus*

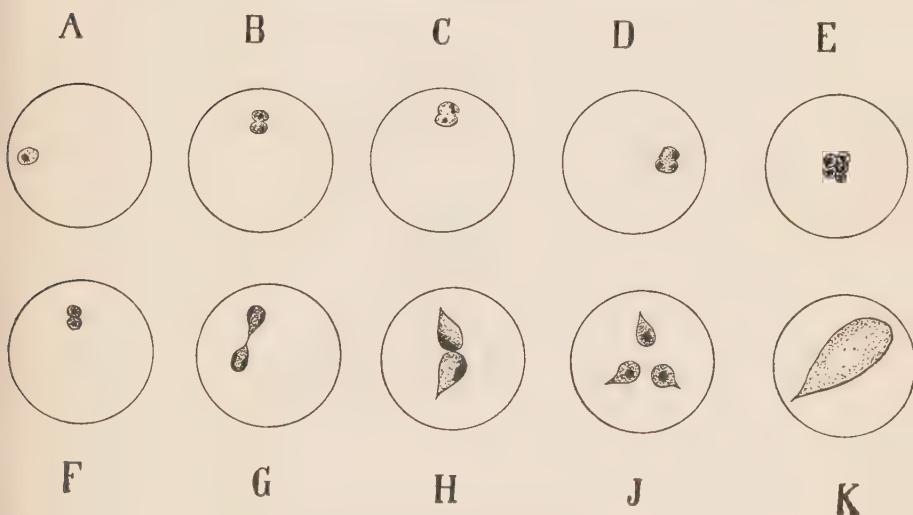


FIG. 60.—PIROSOMA BIGEMINUM. (After Doflein.)

A, Young parasite as seen in the red corpuscles of cattle ; B, formation of the twin-form ; C-E, schizogony ; F-K, development of the larger pear-shaped parasites (? gametocytes).

*bovis* (Fig. 61). The history of the parasites whilst contained within the tick has yet to be worked out, but it may be inferred from the following facts. The disease is not communicated from one infected beast to another, but when cattle have been moved from a part in which the disease is endemic (*e.g.*, Texas and South Carolina in North America) to a region free from the disease no spread of the disease occurs in the cold season, but in

summer epidemics most destructive to the grown cattle occur; in the calves the disease usually runs a favourable course. A minimum period of thirty days is required for the appearance of the disease. The *boophilus* reaches maturity in two weeks, and falls to the ground; there the females produce eggs, which, in from twenty to forty-five days, according to the temperature, produce a new generation of ticks. Careful experiments appear to show that it is this new generation which serves for the conveyance of infection. In this case the ova of the ticks

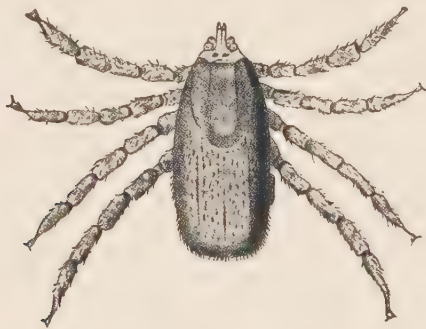


FIG. 61.—*BOOPHILUS BOVIS* (Riley). (After Smith and Kilborne, from Doflein.)

Sexually mature female.

must be infected in a manner partly comparable to the ova of silkworms infected with microsporidia (see below, p. 100). The young ticks seem to be able to live an indefinite period without food, but when they chance to come in contact with cattle they grow and rapidly assume their sexual characters. The tenacity of life exhibited by the young ticks accounts for the long period of time that any pasture remains infected. In fatal cases death may occur in forty-eight hours, but it may be delayed for fourteen days. The destruction of blood-corpuscles is

enormous ; the number of red corpuscles has been known to sink from the normal number (8,000,000 to 9,000,000 to the cubic millimetre) to 31,000 on the second day. The blood plasma becomes of a red colour from the liberated hæmoglobin, and this accounts for the hæmoglobinuria. Sheep, guinea-pigs, rats, and pigeons have proved to be insusceptible.

A parasite very similar to *P. bigem.*, *P. canis*, has been found in dogs ; and Edington\* in South Africa has found another similar parasite in the blood of horses suffering from 'horse-sickness,' a disease so destructive to horses in certain districts of South Africa that animals that have suffered from the disease, and, having recovered, are immune or 'salted,' are worth from six to ten times as much as an animal that has not had the disease.

\* Alex. Edington, *Proc. Roy. Soc.*, vol. lxvii., p. 393, 1900, gives only the general pathology of horse-sickness. His account of the parasites I have not been able to find.

## CHAPTER VI

### NEOSPORIDIA

THREE remaining groups of the sporozoa—namely, the myxosporidia, the microsporidia, and the sarcosporidia—have been brought together into a subclass named the neosporidia. The two former groups, which are closely allied, are by some authors united into one order termed ‘Cnidosporidia,’ because the spores of each are provided with nematocysts or ‘pole-capsules.’ The life-cycle of the neosporidia is still imperfectly known.

#### *The Myxosporidia.*

The myxosporidia consist of a nucleated mass of protoplasm amœba-like in form and motility. The nuclei are usually very numerous. Spore-formation begins in young individuals, and is usually continued for a long period. Each spore is provided with two pole-capsules, and contains a single amœboid germ. The whole animal, which may contain thousands of nuclei and spores, is often several millimetres in length. The formation of spores in these parasites may be regarded as a form of internal budding. Myxosporidia have been found in various classes of animals, from the bryozoa and worms up to the mammalia, but they are most familiar in fishes, and hence were termed ‘fish-psorosperms’ by Müller, who first described them (*Müller's Archiv*, 1851). Thélohan,

in 1894, showed the close relationship of the myxo- to the micro-sporidia, and brought to light many other important features of both groups.

The Cnidosporidia are incapable of a non-parasitic life: they inhabit the cavities of the body in the tissues of their host; only the spores, that are destined to spread the infection, become free, and under favourable conditions develop in the body of a new host.



FIG. 62.—MYXIDIUM LIEBERKÜHNI. (After Balbiani, from L. Pfeiffer.)

*a*, Mature individual containing many spores; *b*, spores: the middle one has its thread-cysts protruded; *c*, young parasites.

The myxosporidia can be somewhat sharply divided into two groups, the first consisting of parasites that inhabit the interior of organs such as the urinary passages, the second of examples that are contained within the tissues. According to Wasielewski, only two intermediate forms are known. The bladder of the common pike invariably contains examples of the species *Myxidium Lieberkühni* (see Fig. 62). According to

L. Pfeiffer,\* in the earlier stages this parasite is contained within the epithelial cells of the bladder. Thélohan, on the contrary, thought that there was no intracellular stage. An example of a microsporidian (*Glugea bryozoides*) is shown in Fig. 66. In this there is a distinct intracellular phase.

The *form* of the myxosporidia is very variable in the stage of adult life. An ectoplasm and an endoplasm can be distinguished; the former, in the free-living forms, is capable of being pushed out into pseudopodia, which subserve locomotion and adhesion to the walls of the cavity that the parasite inhabits. The endoplasm is more coarsely granular than the ectoplasm, and contains many nuclei and spores. Fat-drops are often present, and in some kinds pigmented granules are met with, also vacuoles and hæmatoidin crystals. Like other sporozoa, the cnidosporidia are incapable of taking up solid food: they live by osmotic interchange.

*Reproduction.*—The mode of spore-formation differs from that of the sporozoa described above; instead of a simultaneous process it is a gradual one, beginning in the relatively young animal, and continuing with its growth, the parent parasitic mass retains, meanwhile, its integrity and power of locomotion. The spores are formed by the separation of a sphere of endoplasm around one of the nuclei; this constitutes a *primitive sphere*. The nucleus undergoes karyokinetic subdivision into eight or more nuclei, forming a *pansporoblast* which divides into (1) a thin outer membrane, and (2) two rounded masses (sporoblasts) contained within it. Each sporoblast contains three of the original nuclei of the pansporoblast. The

\* L. Pfeiffer, 'Protozoen als Krankheitserreger,' 1891.

remaining two nuclei, with a little of the cell-plasm, adhere to the outer membrane to undergo disintegration. Each sporoblast then subdivides into three segments, each containing one of the original three nuclei. Two of the segments are smaller than the remaining one, which becomes the germ. The other two soon present each a vacuole, into which a minute knob of plasm soon projects, becoming cut off from the rest of the plasm. It then secretes a membrane, and is drawn out into the spiral, thread-like organ, the nematocyst. The outer layer of each sporoblast becomes modified into a stout spore-shell. The third nucleus—that of the germ—subdivides into two. The primitive capsule is then disintegrated, and the fully formed spores lie free in the endoplasm of free-living parasites and in the interior of a cyst in the tissue-inhabiting one. There is great variety in shape and size of the spores and in the number and disposition of the pole-capsules among the different genera, and on these differences the classification is based: thus (a) the family *Myxididæ* have two pole-capsules and no vacuole in the plasma; whilst (b) the *Chloromyxidæ* have four pole-capsules; (c) the *Myxobolidæ* have a vacuole, the contents of which stain red with iodine, and one or two capsules; and finally (d) the *Glugeidæ*, or microsporidia, have one pole-capsule at their smaller, and a vacuole, the contents of which do not stain with iodine, at their broader end.

As illustrative examples, the *Myxobolus Pfeifferi* (Thélohan) and the *Glugea bombycis* may be considered in some detail.

*Myxobolus Pfeifferi*.—This parasite is frequently met with in apparently healthy fish, causing a usually limited diffuse infiltration (Doflein). In certain rivers—e.g., the

Rhine, the Moselle, the Saar in Germany; the Seine, Marne, and Aisne in France—it has caused widespread destruction of various kinds of fish, but especially of barbel, giving rise to the barbel plague. The diseased fish, as described by L. Pfeiffer,\* are recognised by discoloured swellings of the skin and deep crateriform ulcers on all parts of the body. The contents of the lesions

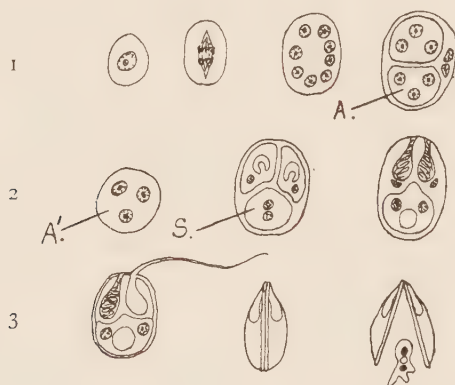


FIG. 63.—SPORE-FORMATION IN MYXOSPORIDIA.

Diagram showing the stages in the myxobolidæ. 1, The nucleus of the primitive sphere undergoes mitotic subdivision till eight nuclei are present, constituting a pansporoblast, which then subdivides into three parts; two segments contain each three, and the remainder, with two nuclei, constitutes a capsule for them. 2, Each of the segments or sporoblasts, *e.g.*, A, A', secretes a cyst, and subdivides into three parts; two of these, each with one nucleus, form the pole-capsules, the remainder, S, becomes the amœboid sporozoite: its nucleus subdivides into two, and a vacuole appears in it. 3, Shows three aspects of the spore; in the third the amœboid sporozoite is escaping.

consist chiefly of the parasites and cell-detritus, with bacteria. The primary seat of the infection which causes the lesions appears to be in the muscle-cells. Similar lesions are present on the peritoneal surface.

The spores, which measure 10 to 12  $\mu$ , are hard,

\* L. Pfeiffer, 'Protozoen als Krankheitserreger,' 1891, p. 105.

shining, and have two pole-capsules, a vacuole, and four nuclei, as shown in Fig. 63).

The manner in which some myxosporidia live amongst the tissues of their host is shown in Fig. 64.

The young parasites in the muscles doubtless develop from the amœboid germ contained in the spore, although the actual passage from spore to muscle-fibre has not been observed. The parasite grows within the muscle-cell until the latter ruptures, allowing the spores to escape into the interstitial tissues, where a sharp inflammatory

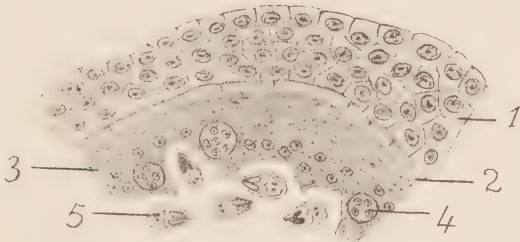


FIG. 64.—MYXOSOMA DUJARDINI. (Thélohan.)

The parasite lies beneath the gill epithelium of fishes. It causes small white, often branched tumours. 1, Epithelium; 2, cortical layer of parasitic mass; 3, deeper layer of parasite containing nuclei; 4, pansporoblast; 5, spores.

reaction is set up, similar to that seen in some cases of sarcosporidial disease in pigs and cattle; but in the fish, owing to the characteristic form of spores, the difference between parasites and leucocytes is more readily seen. This process results in the formation of abscess-like foci, which often undergo secondary bacterial infection, becoming putrid. Severely infected fish may have as many as forty tumours and about one-tenth of the muscle-cells infected. As the result of auto-infection, L. Pfeiffer found that the infection was limited to the musculature; but Doflein observes that the parasites are present to some

extent in almost all the organs, though the infection predominates in the muscles. L. Pfeiffer raises the question whether there is a double cycle of life in the myxosporidia similar to that of the coccidia. More information on this head is called for. Doflein suggests that the severity of the infection is caused by river pollution. He finds that the disease reaches its height in summer and subsides in winter.

The appearance of a barbel severely infected with myxosporidia is shown in Fig. 65.

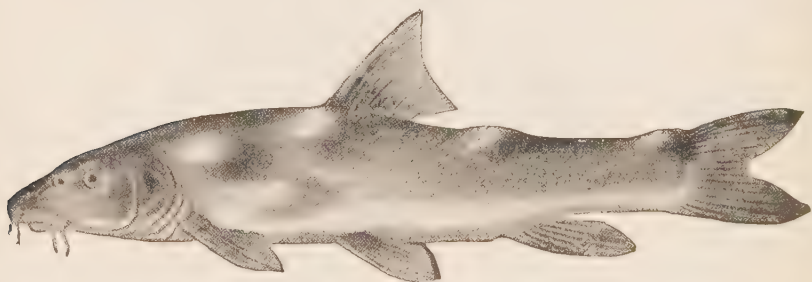


FIG. 65.—MYXOSPORIDIAL DISEASE.

A barbel with multiple tumours caused by *Myxobolus Pfeifferi*.  
(After Doflein.)

### *The Microsporidia.*

In this suborder the spores are small and of a flattened oval or pear-shape. In some a bivalve character of the spore has been observed, as in the spores of the myxosporidia. There is but one pole-capsule, which can only be demonstrated by means of reagents. In some forms the surface of the spore is furrowed. All known forms with one exception are cell-parasites. Their young germs penetrate into the cell-plasm, and there behave like those of the *Plasmodiophora brassicæ*. The nuclei are sharply defined, and the parasites appear to lie naked in the cell-

plasm of the host-cell, Fig. 66; 1, 2, and 3. Nothing is known of any sexual characters.

Sporulation occurs either whilst the parasite is still within the host-cell or when the parasite has ruptured the host-cell and attained a considerable size after its escape. In some instances the infected tissue, together with the parasites, becomes encysted by the formation of a fibrous capsule from the tissues of the host; thus forming cysts which are filled with spores and cell detritus. In other cases, again, there is no such reaction; all the cells of the tissue may be widely infected, and the disease may progress without any inflammation or encapsulation occurring. The pansporoblasts are formed either, as in the myxosporidia, in the interior of the still growing cell-plasma of the parasite, or the latter subdivides completely into numerous pansporoblasts. In the first case each pansporoblast forms very numerous spores; in the latter the number may be very numerous or very few, four to eight. According to the number of spores formed from each spore, the suborder is divided into two tribes:

1. Oligosporogenea (4-8 spores).
2. Polysporogenea (spores numerous and variable) (Doflein).

Two examples, the *Nosema bryozoides* and the *N. bombycis*, both belonging to the polysporogenea, may be studied as examples.

*Nosema Bryozoides* (Korotneff).--This parasite was discovered by Korotneff\* in one of the fresh-water bryozoa, *Alcyonella fungosa*, in the neighbourhood of Moscow. The parasites appear in early summer at the same time as the ripening of the spermatoblasts. At first the parasites can

\* A. Korotneff (Kiew), *Zeitschr. für Wiss. Zool.*, 1892, p. 591.

only be recognised by the microscope, but by the end of July they are recognisable to the naked eye as milk-white opacities. The parasitic masses exert a mechanical effect on their host, causing thinning of the tissues. By August the infected animal constitutes a closed sac distended with parasites, which at this time form large plasmodia either by growth or by fusion. The infection spreads in the colony so widely that hardly a zoid escapes, and the parasites appear to cause the colony to break up much earlier than healthy colonies, which only undergo disintegration at the beginning of winter. The ectoderm cells give way before the pressure of the parasites, the latter then coming into direct contact with the chitinous covering of the host. The stages of the parasite are shown in Fig. 66.

*Nosema Bombycis* (Naegeli) is the sporozoon that determines a destructive disease (*pébrine*) of silkworms, which between the years 1854-1867 cost the silk cultivators of Southern France enormous losses, which were arrested by the practical genius of Pasteur, who instituted methods of distinguishing the infected from the healthy eggs by means of the microscope. The infection begins after an incubation-period of variable duration in the epithelium of the alimentary tract; thence the parasites may penetrate to every one of the tissues of the host, though, according to L. Pfeiffer,\* they appear to have different predilection-sites in different varieties of silkworms. The disease affords a remarkable instance of hereditary transmission. When the disease is not severe the chrysalis stage may be passed, and the female moths may lay fertilized but infected eggs, from which issue weakly, dwarfed caterpillars, which soon

\* 'Protozoen als Krankheitserreger,' p. 135.

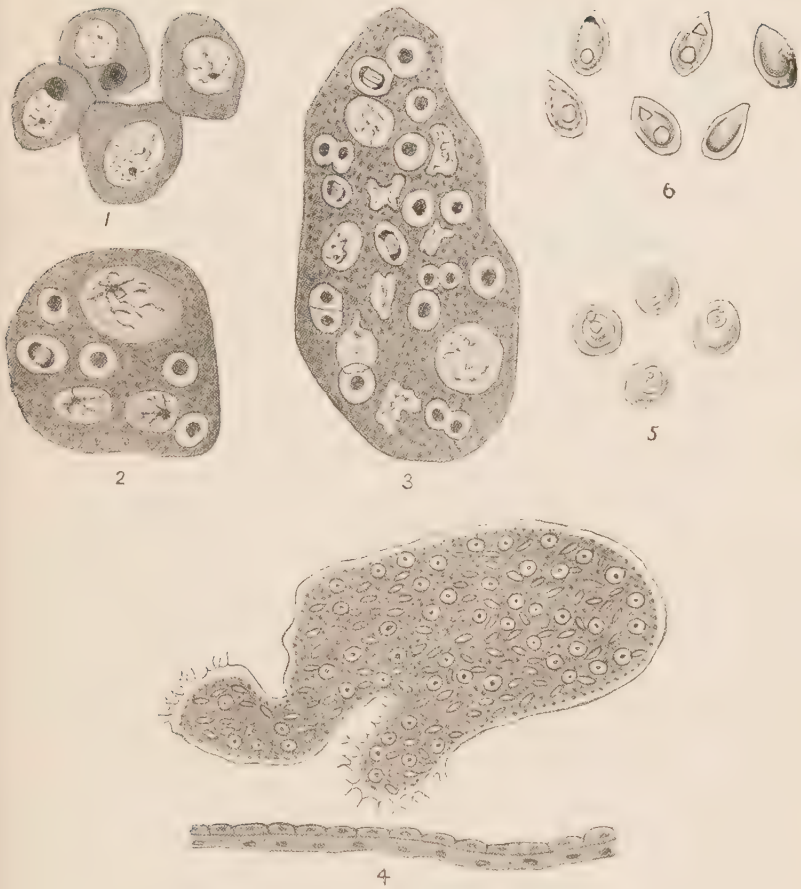


FIG. 66.—NOSEMA BRYOZOIDES (Korotneff).

1, Four spermatoblasts; the two to the left contain close to their nuclei young parasites in which the differentiation of nucleus and plasm has not occurred. 2, A spermatoblast showing a farther stage of the effects of infection; the nucleus has subdivided into three, and there are five parasites, one of which is dividing into two. 3, A still farther stage of cell-infection; the cell is greatly increased in size, and there are multiple nuclei, some of them degenerated. The parasites are very numerous; three are dividing, and several pairs show the result of division. All the above are highly magnified. 4 Shows a large free parasite containing many spores and nuclei; the ectoplasm shows pseudopodia; the double layer of cells belongs to the host. 5 Is an early stage of spore-formation. 6 Shows the completed spores. Both the latter are unstained, and magnified 1,000 diameters. (After Korotneff.)

perish, but not before they may have widely disseminated the disease by means of their dung deposited on the leaves and eaten by previously healthy caterpillars. From each spore taken with the food issues a minute amœboid zoit, which penetrates an epithelial cell (see Fig. 67). The

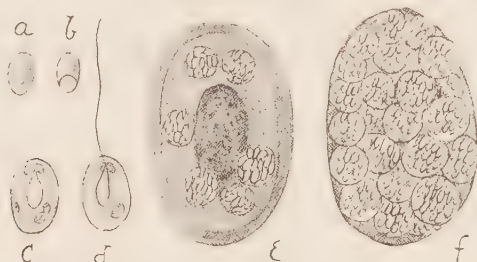


FIG. 67.—NOSEMA BOMBYCIS. (After Thélohan and Balbiani.)

*a, b*, Two spores seen in their natural state; *c, d*, the same spores swollen, and with their pole-capsule and pole-thread visible after treatment with nitric acid; *e, f*, two cells (heavily infected with the parasites) from the alimentary tract of the silkworm.

spores measure 2 to 4  $\mu$  (see Fig. 68); their smallness led Balbiani to apply the term microsporidia to this group of the sporozoa. The *N. bombycis* preys also on two other

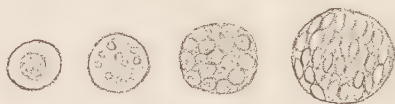


FIG. 68.—NOSEMA BOMBYCIS. (After Balbiani.)

The stages in spore-formation.

moths, *Gastropacha neustria* and the *Saturnia pernyi*. Kindred microsporidia cause tumours in sticklebacks and other fishes.

### *Sarcosporidia.*

These cell-parasites, known since 1843 as 'Miescher's tubes,' were first found in the muscle-cells of mice and

pigs. In spite of a considerable amount of work that has been done of late years with regard to them, the sarcosporidia still remain but very imperfectly understood.

It is sometimes taken for granted that they are harmless, but when their numbers in the muscles of an animal are very great, by reason of their multiplying and causing auto-infection, as is shown in Fig. 69, they produce in the meat an unhealthy gray appearance, which leads to its

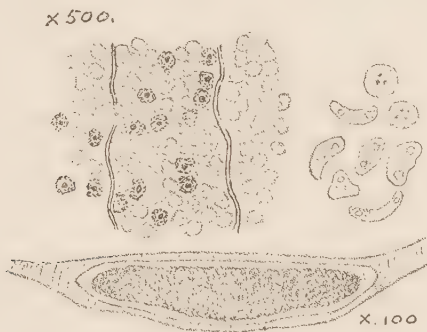


FIG. 69.—SARCOSPORIDIA FROM THE MUSCLE OF A PIG.

Below, a 'Miescher's tube,' or closed sarcosporidial sac.\* Above, to the right, a number of Rainey's corpuscles.\* Above, to the left, part of one of L. Pfeiffer's ruptured sacs; the sarcolemma of the muscle fibre remains; within and on both sides of it are amœboid parasites, some non-nucleated, others more granular and nucleated.

rejection by careful meat inspectors, and the severe degrees of infection have been known to cause paralysis of the respiratory and other muscles; in the former condition death has resulted.

The intracellular parasite commonly has the appearance shown in Fig. 69. The foreign mass lying inside the muscle-fibre is composed of a great number of separate corpuscles, described by Rainey in 1857. Sometimes two

\* From Leuckart's 'Parasites of Man' (English translation).

or more of these parasites are found within the same muscle-fibre. The infected fibre appears to present no change save in the presence of the cavity occupied by the parasite, nor is there any small-cell infiltration around the enclosed parasites. In every case of severe infection, however, examples of L. Pfeiffer's 'ruptured sacs' are to be found. Part of one of these, taken from a section stained with Biondi reagent, is represented towards the upper and left portion of Fig. 69. Instead of the ordinary appearance, the muscle-fibre was reduced to the sarcolemma, and this was ruptured. Instead of Rainey's corpuscles, small bodies of different characters are present within the cavity in the muscle-fibre and among the neighbouring tissues. Most of these amœboid bodies have a delicately reticular structure throughout, and stain blue with the above-named reagent; others are more granular and highly refracting, and have a small nuclear spot dyed green, the remainder taking the orange of the stain, and thus resemble some of the wandering cells found in the connective tissue of cancer. In the neighbourhood of sacs from which most of the amœboid brood have escaped—that is to say, some time after the escape has taken place—some of the muscle-fibres are found to contain groups of nucleated granular cells in their interior. These groups of cells together constitute the early intracellular stage of the parasite. As the latter grows it accommodates itself to the long axis of the muscle-fibre, and the size attained by the parasite depends, as is often the case with intracellular sporozoa, on that of the host-cell. In young intracellular parasites L. Pfeiffer describes a cortical layer which shows slight amœboid alterations in form. The interior of the parasite is first subdivided into round cells,

which in turn break up into sickle-shaped bodies which farther multiply by division, assuming forms which somewhat resemble fission-fungi. No farther change seems to occur within the host-cell.

In pigs, when the number of ruptured sacs is relatively large, the flesh, L. Pfeiffer observes, becomes gray and brittle, and is then unfit for human consumption. The condition had been confounded with actinomycosis until the above-named author pointed out the distinction. After all the sickles have escaped, the vacated sac becomes filled with degenerate material, and sometimes calcified, and, surrounded by the amœboid parasites, it resembles roughly in transverse sections a clump of actinomyces surrounded by small and endothelioid cells. L. Pfeiffer has named the condition *Myositis sarcosporidica*.

In horses the same progressive infection occurs, and is fully described by L. Pfeiffer. At an early stage the affected spots look as if they were the seat of inflammatory changes; in cases of longer standing distinct tumours are formed, the 'Muskel-knospen' of Kölliker. These tumours, the real nature of which L. Pfeiffer has made clear, in sections strongly resemble small-celled scirrhus cancers, the parasitic cells looking like small epithelial cells, and lying in spaces bounded by thick bands of fibrous tissue.

In the œsophagus of the sheep these parasites cause the formation of cysts, varying in size from a millet-seed to a pea. The centre of the older cysts is occupied by degenerated colloid material; the parasites lie next the capsule of the cyst, each succeeding brood works out into the surrounding muscular fibres, and thus furnishes a type of a lesion progressing serpigginously with central degeneration.

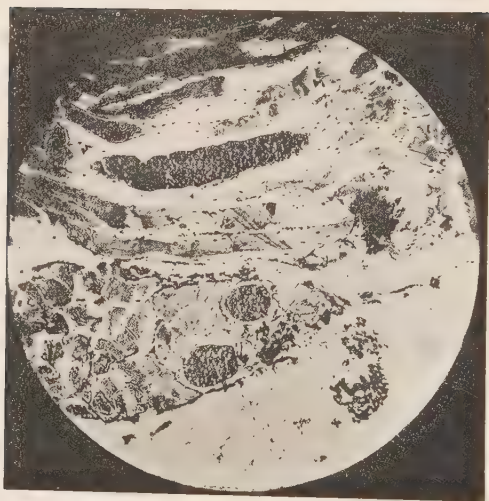


FIG. 70.—SARCOSPORIDIA OF PIG'S MUSCLE (Longitudinal Section).  
(After L. Pfeiffer.)

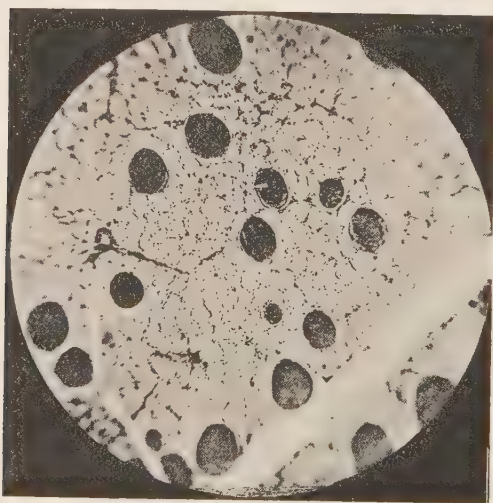


FIG. 71.—SARCOSPORIDIA OF PIG'S MUSCLE (Transverse Section).  
(After L. Pfeiffer.)

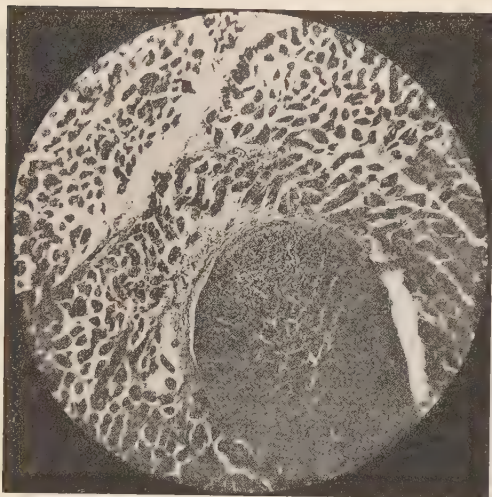


FIG. 72.—SARCOSPORIDIAL CYST-FORMATION IN THE SHEEP'S ŒSOPHAGUS. (After L. Pfeiffer.)

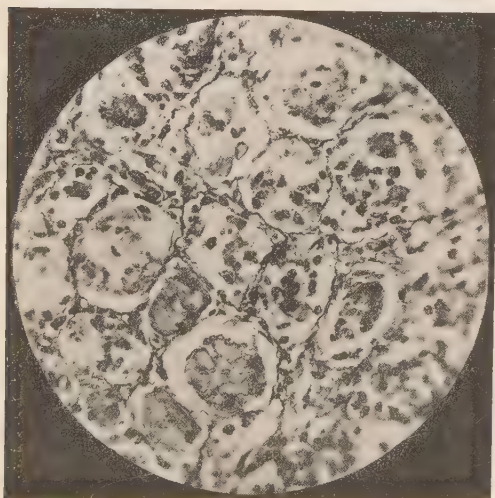


FIG. 73.—FIBROSED SARCOSPORIDIAL NODULE IN HORSE'S MUSCLE. (After L. Pfeiffer.)

These facts, as well as the ordinary histological appearances, are shown clearly in the accompanying Figs. 70-73, taken from one of the works of L. Pfeiffer,\* who points out close similarities between the mode of tissue invasion of all the neosporidia, and also of cancer.

In the sheep, goat, horse, and kangaroo sarcosporidial tumours or cysts (see Figs. 72 and 73), from the size of a bean to that of a hazel-nut, are of common occurrence. L. Pfeiffer (1895) has shown, and Laveran has since confirmed this observation, that a glycerin extract of the sarcosporidial cysts of the sheep's œsophagus is intensely toxic to rabbits, causing intense local inflammation and a general febrile reaction, which has been compared to the reaction of tuberculous animals to tuberculin.

The principal features of these organisms may now be recapitulated. They first show themselves as small round or elongated sacs. When full grown they are often readily visible to the naked eye, and can easily be isolated by teasing. The larger sacs have a double contour. The outer sheath is striated vertically to the surface. This striation may be due either to the presence of rodlike structures or to the presence of minute canals, the latter hypothesis being the more probable. The inner membrane is thin and hyaline; the two layers together appear to constitute the ectoplasm. In the endoplasm even of the small parasites rounded nucleated bodies from 4 to 5  $\mu$  in diameter are differentiated, see Fig. 74, A. They are comparable to the pansporoblasts of the cnidosporidia. The protoplasm in which the pansporoblasts are embedded forms an alveolar meshwork, from which

\* L. Pfeiffer, 'Der Parasitismus der epithelial Carcinoms, sowie der Sarko-, Mikro-, und Myxo-sporidien in Muskelgewebe,' 1893.

the pansporoblasts may be removed without destroying its structure. When the sac has reached a certain size the nuclei of the pansporoblasts divide, and whilst this is taking place in the central parts of the sacs new pansporoblasts are being formed towards their ends, prob-

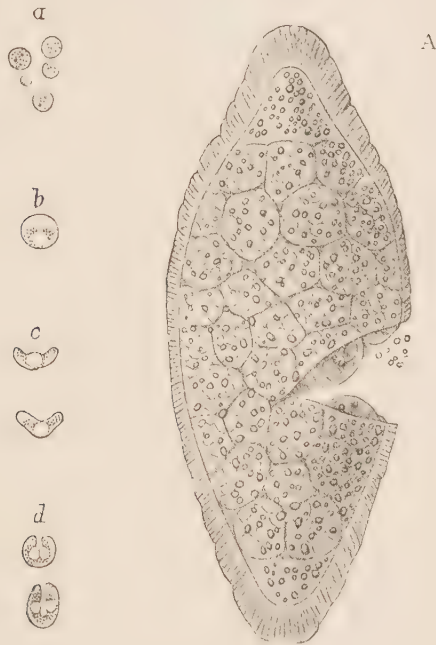


FIG. 74.—*SARCOCYSTIS MIESCHERIANA* (Kühne). (After Manz, from Wasielewski.)

A, Full-grown 'Miescher's tube' separated from its host muscle-fibre; on the right side the radially striated ectoderm is ruptured, showing the pansporoblasts. *a-d*, Development of the spores from the sporoblasts.

ably by division of adjoining pansporoblasts (Doflein). Spore-formation takes place from the multinucleated pansporoblasts by their separation into rounded uninucleated segments, from each of which forms a single spore. The shape of the spores may be modified by mutual

pressure, but they tend to be kidney- or sickle-shaped. They vary from 3 to 12  $\mu$  by 1 to 5  $\mu$ . At one or both ends the spore may have one or two whiplike structures, which have been compared to the pole-capsules of the cnidosporidia, but since both sporoblasts and spores are uninucleated, it hardly seems probable that the thread-like appendages have the same significance as the nematocysts.

The systematic subdivision of the sarcosporidia is necessarily imperfect, but provisionally the genus *Sarcocystis* (Ray Lankester) is taken by Doflein to include the common sarcosporidia of the pig (*Sarcocystis Miescheriana*); the *S. Bertrami* of the muscles of the horse; *S. tenella* of the sheep; *S. Blanchardi* of the muscles of the ox; *S. Lindemanni* of the muscles of man; and the *S. hueti*. The last was found in great numbers in the muscles of a seal; it differs somewhat from those of the other species of sarcocystis; the spores are spindle-shaped or reniform.

#### *Serumsporidia.*

L. Pfeiffer\* has described a series of unicellular parasites that he found in the lower crustacea, such as cypris, daphnia, cyclops, and gammarus (the fresh-water shrimp), and has suggested for them the above name. More recently, G. N. Calkins† has referred to the same category certain parasites that he has discovered. The material was afforded by an epidemic among the brook-trout (*Salvelinus fontinalis*) of a trout farm in Long Island. The disease was equally fatal to fish of all ages. The affected fish were sluggish and speedily

\* L. Pfeiffer, 'Protozoen als Krankheitserreger.' Nachtr  g, 1905.

† G. N. Calkins, 'Report upon the Recent Epidemic among Brook Trout on Long Island,' *Fourth Annual Report New York Commissioners of Fisheries*, etc., 1898.

perished when transferred to a pail. They frequently rose to the surface and turned on the side or belly upwards. Hundreds of dead fish were at one period removed daily from the runways. In some there was no external appearance of disease. In others discoloration and red ulcers were present on various parts of the body, especially on the sides. The eyes and jaws were frequently affected; in some of the deeper ulcers the vertebræ were exposed. On investigating the outbreak, Calkins came to the conclusion that it was caused by a previously undescribed sporozoon, to which he gave the name *Lymphosporidium truttæ*. Professor Calkins having kindly furnished me with sections and tissue, I have been able to confirm the description of the pathology of the disease that he has given in a small monograph, extracts from which may be advantageously quoted in this place.

*Lymphosporidium Truttæ* (Calkins).—Sections of tissues fixed in acetic acid sublimate solution, and by other means and stained with thionin, etc., showed the parasites in the lymph-spaces of all parts of the body; thus, on the surface of the intestines, liver, etc., and in the intermuscular lymph-spaces, were clusters of innumerable, roundish, pyriform bodies; when stained they are of homogeneous appearance and measuring from 2 to 2.5  $\mu$ . The spleen and kidneys contained a few of these bodies scattered in their substance, but the testis was the only organ which contained them massed together in countless numbers. The path of infection appeared to be by the alimentary tract, for in the intestine they were present in considerable numbers in various stages of development. These small bodies Calkins terms 'spores.' 'The spores are pyriform in shape, but flattened on the broader end. Under ordinary

conditions of fixation and staining they appear to be homogeneous and without internal structure of any kind; they always stain intensely with the nuclear stains (basic stains). I was unable to determine whether the homogeneous appearance indicates a similar condition throughout the entire cell, or whether it was due to incomplete extraction of the stain. In many cases, however, preparations were obtained in which the organism was differentiated into a peripheral, deeply staining portion and a less stained part with a central nucleus-like body. This condition, however, marks a stage in the life-history and indicates the preparation for sporozoit formation, while the intense homogeneous appearance indicates a young form or an unripe spore. . . . Great bunches of spores, as described above, are found in the intestine, and in such groups there are, here and there, certain individuals in which the body is divided up into eight parts. These parts are the sporozoits, and in some cases, in certain divisions of the intestine, all the individuals of a group are in some stage of sporozoit formation. The first indication of the process is a noticeable cleft beginning at the broad end of the spore, while the entire periphery appears irregular and minutely lobed. The mass of protoplasm segregates into eight small spheres, which are not confined by a membrane. The method of formation of these reproductive bodies could not be determined owing to the extremely minute size, although various stages were seen and it was conclusively proved that the groups of eight spheres were derived from single spores. Each sporozoit at this stage measures less than half a micron.'

Similar processes were observed in the testis, but here

the sporozoits were contained within a membrane which was wanting in the intestine, having probably been dissolved by the digestive juices. No nucleus was made out in either spores or sporozoits, the chromatin being probably distributed throughout the organisms. Calkins found sporozoits in the interior of epithelial cells of the stomach and intestine. As they increase in size they attain the dimensions of the spores, from which, however, they are distinguishable by assuming amœboid forms which were never observed in the spore. In the amœboid form the young parasite penetrates between the unstriped muscle fibres of the intestinal wall, becoming first vacuolated and then reticulated in structure as it grows. The largest intramuscular amœboid forms measure from 18 to 25  $\mu$ ; they are elongated in shape, and in their latest stages they become densely granular; the granules react to stains like chromatin, but the granular parasites are distinguishable from the nuclei. 'Spore-formation is always preceded by encystment of the animal within a delicate membrane. The cell leaves the muscle tissue, and in the lymph of the body-cavity it rounds out into a sphere. The amœboid individuals, when ready to form spores, are comparatively large, and the cysts are of variable size, in some cases measuring 20 microns or more in diameter. The spores are formed by aggregation of the deeply staining granules (chromatin?) instead of by nuclear division as in other sporozoa. This leaves the protoplasm with a clearly marked reticular structure, as in the earlier stages (Fig. 75). A variable number of spores is the rule. In some cysts twelve were seen, in others sixteen or even more. In some cases the cysts appear to be differentiated into a more hyaline ectoplasmic and a denser endoplasmic region . . .

a more or less definite membrane separating the two. This membrane is not obligatory, however, and is often wanting. The spores thus formed are liberated into the

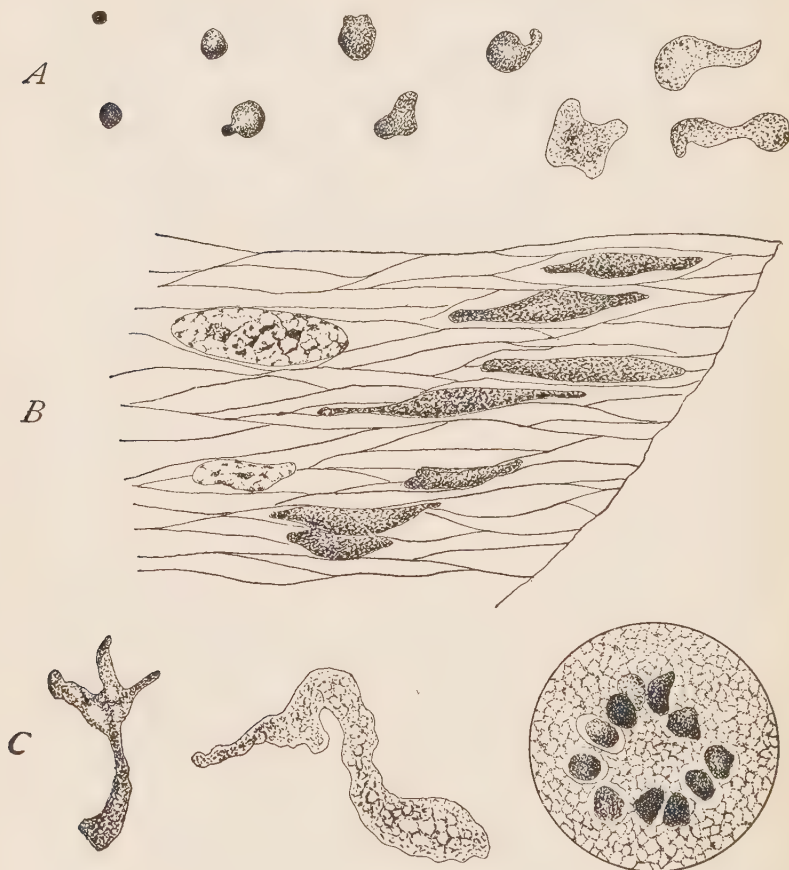


FIG. 75.—LYMPHOSPORIDIUM TRUTTÆ. (After G. N. Calkins.)

A, The young sporozoite and its development ; B, older forms in the muscle bundles surrounding the intestine ; C, still older amœboid forms prior to and during spore-formation.

body-cavity and carried to all parts of the body with the lymph and blood.' Not only on account of its being new to biology and pathology, but also by reason of its pecu-

liarities of structure,\* is the *L. truttae* of Calkins of great interest, and the salient features of its life-history are well worthy of recapitulation :

1. The parasites are ingested with the food either in the cyst (adult) or the spore stage.

2. The spores liberate sporozoits in the intestine.

3. The sporozoits penetrate the epithelium and work their way to the lymph-spaces.

4. In the lymph they develop amœboid processes and penetrate muscle bundles.

5. In the muscles they grow to adult size, becoming large amœboid organisms of spindle or club shape.

6. They return to the lymph, and become rounded into spore-forming cysts.

7. The spores are apparently formed by the segregation of chromatin (?) granules ; they vary in number.

8. The spores are liberated in the body-cavity, from which they find their way to all parts of the body, but accumulate especially in the testis. Or the cysts may be voided to the outside by way of the gall-bladder.

9. In the testis these spores form sporozoits, and thus lead to auto-infection. These spores, unlike those in the intestine, are contained in a capsule.

*Haplosporidia*.†—This order has been formed to include examples of protozoa of as yet insufficiently well-defined characters. The members of the group are polynuclear, and multiply by uninucleated spores, which recall the spores of the microsporidia, but are devoid of a pole-capsule.

\* These should be compared with various bodies suspected of being parasitic protozoa in small-pox and other diseases.

† Caullery and Mesnil, *C. R. Soc. Biol.*, Paris, ser. xi., tom. i, 1899, p. 791.

## CHAPTER VII

### THE FLAGELLATA

A SIMPLE flagellate has a more or less oval body, which at one end is prolonged into a filament, or flagellum. In progression this filament is always in front, and hence the end of the animalcule from which it springs is termed 'anterior.'

Near the point of attachment of the flagellum is a depression in the body of the animal, the mouth and pharynx; the aspect on which the mouth is placed is termed 'ventral.'

The cytoplasm contains food-granules and some granules suspended in a homogeneous blind endoplasm. The ectoplasm constitutes a thin layer beneath the cell-membrane.

The nucleus is round, with a definite, though delicate, nuclear membrane. The nucleolus is surrounded by what usually appears to be a clear space, but in which a delicate network may sometimes be made out.

The flagellata are aquatic, and move both actively by general changes in the body-form, but especially by virtue of the function of the flagellum. The latter is always longer than the body of the animalcule, and it moves in such a way that it draws the body after it; thus its action

is the opposite to that of the tail of a spermatozoon, which propels the body of the cell before it. The flagellum plays a part also in the capture of food, which for most flagellates consists of bacteria, etc., which by the action of the flagellum are thrown into the pharynx, and there are rapidly taken into the protoplasm of the body.

The flagellum is not always single; many flagellates have two, which may be equal in length and both directed forwards, or one forwards and one backwards, as in *Bodo*; or they may be unequal. Sometimes the flagella are numerous, in which case they may be distributed about the animal's body, or clustered at certain points. The origin of the flagellum in some instances has been traced to the close proximity of the nucleus. In the trypanosomata it appears to be closely associated with the function of nuclear division (see below, p. 124).

The endoplasm is the seat of irregular oscillations. Excretion is effected by a contractile vacuole.

Protection cysts are formed when adverse conditions, such as dryness or an excessive putridity of the water, supervene. The animalcule loses its flagellum, becomes rounded, and secretes a membranous cyst, from which it escapes when more favourable conditions recur.

*Reproduction.*—In the free state longitudinal division is the rule among the flagellates. The nucleus first divides by a simplified mitosis, and a second flagellum, mouth, and contractile vacuole form, and the parasite then divides, beginning at the anterior extremity. An inexperienced observer may readily mistake a longitudinal for a transverse division (see Fig. 76). In the dinoflagel-

lata division is oblique, and in two other forms\* transverse division has been observed.

*Division under a cyst* often occurs; the organism becomes rounded, loses its flagellum, and secretes a cyst, as in protective encystment. Division into two, four, or many ensues. The young brood are liberated by rupture of the cyst.

*Conjugation* is observed among the flagellata: 'Two individuals approach and adhere together, lose their flagella, and assume a more or less amœboid character, and by degrees undergo fusion one with another; their nuclei also fuse together. It is a total conjugation. The



FIG. 76.—LONGITUDINAL DIVISION OF A FLAGELLATE. (After Delage and Hérourard.)

The stage before separation simulates transverse division.

resulting conjugate individual becomes encysted, and in this state divides into numerous spores.

All these events have not been observed in every species, and in some species other phenomena occur. The most important of these are the formation of little receptacles for the protection of the individual, the formation of colonies by the association of individuals which either remain naked or secrete a gelatinous substance in

\* These are the chlamydomonadina and the volvocina, both of doubtfully animal characters. In one colony-forming flagellate, *Codosiga botrytis*, the usual form of division is longitudinal, but transverse division resulting in the formation of a free daughter-cell has also been described (see Lang, 'Protozoa,' p. 172).

which they are embedded. The flagella may be multiple (from two to eight). When this is the case, at least one flagellum is directed forwards. Many flagellate organisms contain chlorophyll or diatomine, and live like plants. One of these, *volvox*, forms a colony, in which differentiated sexual elements (micro- and macro-gametes) are developed, and fuse together to form zygotes, which become encysted and subdivide into spores. The chief subdivisions (or subclasses) of the flagellata, according to Delage and Hérouard, are :

1. Euflagelliæ—simple flagellates, such as are exemplified by the 'type' already described.
2. Dinoflagelliæ, which are protected by cuticular plates; and
3. Cystoflagelliæ, of which *noctiluca* is the better-known genus.

The two latter forms are relatively large, measuring 1 millimetre in diameter, and being of free pelagic habit, they need not be farther discussed here. The euflagelliæ, on the contrary, include parasitic and pathogenic forms, such as the *Trypanosoma Brucei*, which causes the tsetse-fly disease; and several other genera demand notice on account of their pathological or other peculiarities.

*Mastigamæba* has a single flagellum, and when swimming appears like an ordinary flagellate, but when feeding its progress becomes slower, and it puts out from all parts of the body pseudopodia, which capture food exactly like an amœba. It forms a link between the rhizopoda and the flagellata.

*Dimastigamæba* resembles the preceding, but is furnished with two flagella. This genus can incept food both as a flagellate and as an amœba.

*Cercomonas* bears a single flagellum at its anterior extremity, whilst its opposite extremity is prolonged into a long, pointed, caudal appendage. Pseudopodia, especially during conjugation, may form at the base of this appendage. The occurrence of this genus in the dejecta of certain cases of diarrhoea of man makes it worthy of mention.

Next to the sporozoa, the flagellata are the protozoa that are most likely to cause disease. Their manifold life-history, including encystment and varied reproductive processes, may serve to render their detection difficult in morbid tissues. It has already been mentioned that flagellates are the first protozoa to appear in infusions, and hence care must be taken not to confuse merely saprophytic with pathogenic species. Detached ciliated epithelial cells have been mistaken for flagellate protozoa. Simple flagellates—e.g., *Cercomonas intestinalis* (Davaine, 1854) and *Trichomonas vaginalis* (Donné, 1837)—have been observed in man; another, *Herpetomonas muscae domesticæ*, in the intestine of the house-fly. L. Pfeiffer found myriads of flagellates in the so-called diphtheria of birds. The chief interest at present is centred in the trypanosomata, which are characterized by the *undulating membrane* which extends along one side of the animal's body, and which has at its free border a prolongation of the flagellum.

*Trypanosoma*\* *sanguinis* occurs free in the blood-plasma of various kinds of frogs (*Rana temporaria*, *Rana Esculenta*, *Hyla arborea*). It is characterized by a broad body, which is often curved upon itself, and measures 40 to 80  $\mu$  long

\* So named by Gruby in 1843 on account of its auger- or trephine-like movement.

by 5 to 10  $\mu$  broad (Doflein). Both the body and the undulating membrane of this parasite are broader than in most other trypanosomata. The mode of transference from one host to another is unknown.

*Trypanosoma Lewisi*.—This flagellate occurs as a parasite in the blood of rats. It has been found in many parts of Europe, including England, in Asia, and in Africa.\* The body of the parasite is elongated, measuring with its flagellum 25  $\mu$ . The endoplasm is finely granular, and, as in most flagellates, the ectoplasm, though thin, constitutes a definite layer. The flagellum is about as long as the body, and from the attachment of its free part to the anterior end of the parasite it can be traced as the somewhat thickened border of the undulating membrane to the posterior end of the parasite, to terminate in a highly-refracting spot situated in the body when the latter begins to taper to a point. In fresh blood the parasites are highly mobile, and the flexibility of their bodies gives them a vermicular appearance. The parasite is never found in the interior of the red blood-corpuscles.

*Symptoms in the Rat*.—All observers agree that, although the number of parasites may be very great, the rats appear to be but little inconvenienced. Laveran and Mesnil,† on whose work the following account is largely based, found in a set of young rats where the infection was unusually intense (three trypanosomes to every red blood-corpuscle) the rats ceased to put on weight during the first week

\* Lewis at Calcutta found the sewer rat (*Mus decumanus*) and the brown rat (*M. rufescens*) were affected in the proportion of 29 per cent. Crookshank, in London, found 25 per cent. of sewer rats infected. Laveran in Paris found only two out of forty-three sewer rats were infected.

† Laveran and Mesnil, *Ann. de l'Inst. Pasteur*, September 25, 1901.

of the infection. There appears to be no febrile reaction.

*Transmission of the Disease.*—Laveran and Mesnil found living parasites in the altered blood in the stomach of a flea taken from the fur of an infected rat, and they also succeeded, in five instances out of nine, in conveying the disease by means of blood obtained by crushing several fleas, and diluting this with saline solution and injecting it into the peritoneal cavity. Thus, it appears probable that fleas are the agents in disseminating the infection in natural conditions. The young of an infected mother are free from the parasites; thus, the placental filtration appears to be an effectual barrier to the passage of the parasites. L. Rogers observed that by eating the bodies of dead rats containing trypanosomes other rats were only infected if the mucous membrane of their mouth had been previously injured.

Experimentally, white and pied rats (*M. rattus*) proved the most useful. White mice were immune, and guinea-pigs almost so. The most certain mode of infection is by intraperitoneal injection of blood containing the trypanosomes. Subcutaneous injection is also successful, but the appearance of the parasites in the blood is delayed.

*Course of the Infection.*—Laveran and Mesnil found that after intraperitoneal injection multiplication of the parasites began in twenty-four to thirty-six hours, and reached its maximum on the third day, and ceased soon after. This constitutes the first period of infection. Parasites may be found in the blood five or six hours after intraperitoneal inoculation, when young, and hence small rats are used, but it is usually during the fourth day that the parasites are numerous and multiplying in the blood.

This constitutes the *second period* of infection, which lasts to the eighth day, or thereabout. After this, for from twenty days to two or four months, only adult trypanosomes are found (usually one to two or three red blood-corpuscles), and the multiplying forms are no longer met with. This is the *third period* of infection. After the parasites have disappeared from the blood the animal is found to be immune. In old rats the infection is often less intense and its duration shorter (eight to ten days).

*Period of Survival of the Parasites.*—In blood that has been drawn from the body of an infected rat and kept in a glass tube most of the parasites die within four days in summer, whilst in winter, under the same conditions (except for the lower temperature), they may remain alive for eighteen days. In an ice-safe they live much longer, from thirty to fifty-two days. Infected blood that has been kept so long that no recognisable parasites are to be found in it may still be capable of conveying the infection, but with a lengthened incubation-period.

*The Morphology of the Parasite.*—In the fresh state the parasites may be studied either in the same way as malarial blood or in the hanging drop, the blood being readily obtained from the rat's tail. The movements of the red blood-corpuscles betray the presence of the parasites under a moderately low power.

For stained preparations Laveran and Mesnil recommend that a drop of blood be spread in a thin layer on a slide, rapidly dried, and then fixed in absolute alcohol. For staining, some authors have used modifications of Romanowski's stain; Mesnil and Laveran found the one given below\* to give the best results.

\* See Appendix.

For a rough examination an alcoholic solution of fuchsin or carbol-thionin may be used. When Romanowski's or Laveran's method is used the protoplasm of the parasite stains blue, the nucleus, flagellum, and its continuation along the undulating membrane and the corpuscle near its termination in the body of the parasite are violet. The membrane itself is not stained.

*The Mode of Division of the Parasite* is by longitudinal division. Laveran regards the corpuscle at the end of the

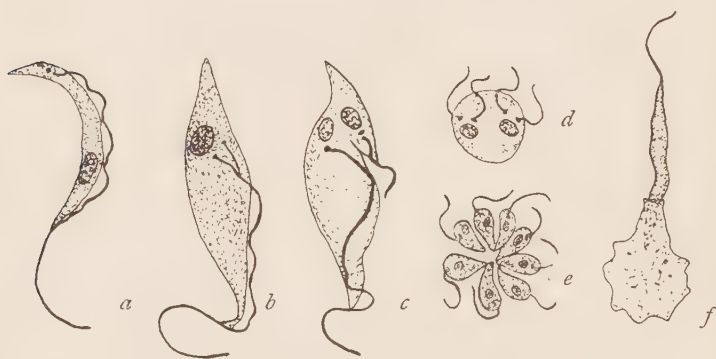


FIG. 77.—*TRYPANOSOMA LEWISI* FROM RAT'S BLOOD. (After Laveran and Mesnil.)

*a*, Adult parasite; *b*, *c*, stages in division; *d*, *e*, forms due to multiple divisions; *f*, phagocytosis—a leucocyte in process of surrounding a trypanosome.

flagellum as a centrosome. Before dividing the body of the parasite the nucleus and the centrosome increase in length and breadth; the base of the flagellum becomes thickened, and the nucleus and centrosome approach each other. The nucleus may divide before the centrosome, or *vice versa*; the thickened base of the flagellum also divides (see Fig. 77). Finally, the protoplasm divides. If the division of the protoplasm is delayed, multi-nucleated and multiflagellate masses are formed. In-

volution forms readily occur in blood that has been kept for some time.

*Agglomeration of Parasites.*—Laveran and Mesnil have made a series of careful observations, from which it would appear that phenomena similar to those observed in the

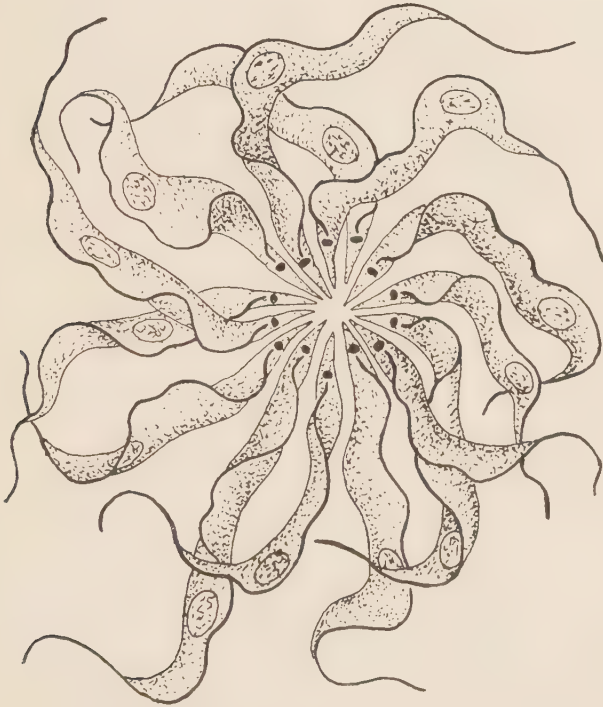


FIG. 78.—*TRYPANOSOMA LEWISI*. (After Laveran and Mesnil.)

A rosette formed by agglomeration of fourteen parasites.

case of typhoid and other bacilli occur in trypanosomes : (a) in defibrinated blood kept on ice ; and (b) when defibrinated blood containing trypanosomes is mixed with serum of rats rendered immune by successive intra-peritoneal injections of the parasites. Agglomeration

begins by two parasites adhering at their posterior ends; their flagella remain active. Rosettes are formed by additional parasites joining the original pair (see Fig. 78). Sometimes the parasites succeed in detaching themselves. Powerful serums cause compound rosettes. The phenomenon is attributable to the presence of an agglutinine in the serum. The serum of fresh rats is not agglutinant. Parasites killed by chloroform or formol are agglutinated, but without any arrangement in pairs or rosettes.

*Immunity.*—Rats protected by previous infection possess an active immunity. The serum of such a rat, especially if fortified by repeated injections of the parasite, will confer a *passive immunity*, which in most cases lasts for over two weeks. The parasites are destroyed by phagocytosis. The leucocytes capture the parasites in full movement in immune animals.

### *Tsetse-fly Disease or Nagana.*

Travellers in Central and Southern Africa\* have long since made the world familiar with an affection fatal to horses and cattle due to the bite of a fly (Fig. 79) which was harmless to human beings. David Bruce,† in the course of an investigation carried out in Zululand, discovered the cause of the disease to be, not, as was previously supposed, a poison secreted by this fly that has given its name to the malady, but a flagellate protozoon (*Trypanosoma Brucei*), similar in appearance to that already described as occurring in rats, the part played by the flies would seem to be merely that of carriers and

\* *E.g.*, Dr. Livingstone, 'Missionary Travels and Researches in South Africa,' 1857.

† D. Bruce, 'Preliminary Report on the Tsetse-Fly Disease,' 1895.

not intermediate hosts as are the mosquitoes that spread malaria.

The latest account of this disease is one by Laveran and Mesnil in the *Annals of the Pasteur Institute* in Paris, January 25, 1902. This account, which is easily accessible, gives full references to previous work done on

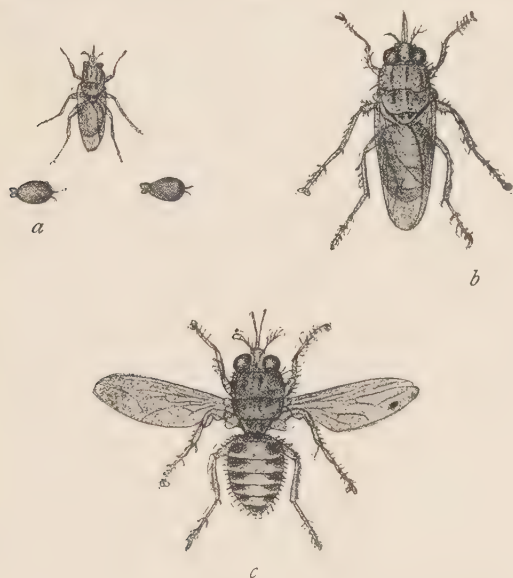


FIG. 79.—THE TSETSE-FLY. (After Bruce.)

*a*, Female (natural size) with two larvæ produced viviparously; *b*, female with closed wings; *c*, with opened wings (*b* and *c* twice natural size).

the subject; hence, as in the case of the flagellates of rats, a detailed list of literature is unnecessary.

Another disease occurs in Northern India and neighbouring parts of Asia in horses, mules, and camels. It is known as 'surra.' In this affection Evans\* discovered a flagellate, and this discovery was confirmed by Steel.

\* Evans, 'Report on Surra,' 1880.

Crookshank described the parasite, and demonstrated it to be a flagellate protozoon, which was named *T. Evansii*. The question has arisen as to the identity of tsetse-fly disease and surra. R. Koch, Nocard, and L. Rogers have expressed the opinion that the affections are identical, but before this can be regarded as settled further observations are desirable; for whilst for horses, mules, asses, goats, sheep, dogs, rabbits, guinea-pigs, and rats, the two diseases have been found to be equally fatal, a difference has been observed with regard to cattle. Whilst cattle, according to Bruce, nearly always die of tsetse-fly disease, they usually recover from surra. The general symptoms of the two diseases are much alike in animals of the same species. In camels surra may have a course prolonged for three years. This, however, can hardly be regarded as a point of difference, because the large game animals, buffaloes, antelopes, etc., appear not to betray symptoms of the disease, though they seem to be the source from which the tsetse-fly obtains infected blood. Bruce observes that the disease disappears with the large game. It would thus appear that they, too, have it in a chronic form. The probability is that tsetse-fly disease and surra are the same.

The mode of dissemination of the infection has already been referred to. In Zululand Bruce came to the conclusion that the tsetse-fly was the only one capable of carrying the disease. In India L. Rogers has found that other blood-sucking flies serve for the dissemination of surra.

The power of infection remains with the fly for from twelve to twenty-four hours as a rule; after forty-eight hours it becomes very uncertain.

*Symptoms.*—The course of the disease differs in the various species of animals. The number of parasites found in the blood is variable. Laveran and Mesnil found that in rats and mice they may be more numerous than the red corpuscles, whilst in rabbits, guinea-pigs, and sheep it is exceptional to find the parasite on microscopic examination of the blood. Infected rats and mice show no symptoms until just before they die. Mice usually die without showing anything but erection of the hair and slight dyspnœa; rats are convulsed a few minutes before death. After peritoneal inoculation rats die in two, mice in three days. After subcutaneous inoculation in these same species of animals death occurs in from three and a half to five and a half days. Healthy rabbits live from twelve to forty days after inoculation, and the blood contains but few parasites; it serves to convey the disease to mice and rats, the period of incubation being from four to six days.

In dogs Bruce\* found that the disease is rapid and invariably fatal. The chief symptoms are extreme emaciation, swelling of the extremities, eruption over the body with the formation of blebs and pustules containing more or less purulent matter, and finally milky opacity of the cornea, giving rise to blindness. In two cases related by Bruce the temperature went up to over  $105^{\circ}$  F. from the normal  $100^{\circ}$  F.

In the horse the first appearance of illness is a 'staring' of the coat, and there is a watery discharge from eyes and nose; shortly afterwards a slight swelling under the belly. Fugitive swellings appear in the hind-quarters. The

\* D. Bruce, 'Further Report on Tsetse-Fly Disease, or Nagana,' May, 1896.

animal becomes progressively emaciated, looks dull, and hangs its head. Then swellings under the skin appear, and the animal may become blind. At the last the victim falls down and dies of exhaustion. The temperature may reach 107° F. from a normal of 100° F.; the course is from three weeks to a month. In the donkey the course is much the same as in the horse. In cattle the course of the disease may be much slower than in the horse; many die within a month, others linger on for six months or longer—only a small percentage recover.

The only post-mortem appearance that is at all constant is swelling of the spleen; the lymphatic glands are also swollen sometimes.

*Trypanosoma Brucei.\**

In fresh blood under the microscope the parasite appears as a mobile vermicular body with an undulating membrane and flagellum. The movements soon grow slower in ordinary preparations, and then the wave-like movements of the membrane are readily seen. The posterior extremity of the parasite is sometimes pointed, sometimes blunt. In well-stained preparations Laveran and Mesnil found that the parasites are of the same dimensions in various kinds of animals, though from the varying size of the red blood-corpuscles in different animals they may appear to be larger or smaller by comparison.

As compared with *T. Lewisi*, these parasites are usually distinguished by the more granular appearance of the protoplasm of the anterior half of the body. In prepara-

\* In this account Laveran and Mesnil's description is followed.

tions stained by Laveran's method (see Appendix) they take a deep blue colour. The posterior extremity of the parasite is often blunt. The nucleus is elongated, and it contains numerous granular masses of chromatin. The corpuscle at the beginning of the flagellum in the posterior part of the body stains deeply, and is usually surrounded by a small clear zone.

Bruce observed that these parasites increased in numbers by longitudinal division. Kanthack, Durham, and Blandford regarded small oval bodies, with or without flagellum, that they found in the lymphatic glands, as the young forms of the parasite. Laveran and Mesnil say that the dividing forms of these parasites are more easily found in the blood than are those of *T. Lewisi*, because in the case of the latter the period of reproduction in the blood is restricted.

The general plan of division (Fig. 80) is the same as in *T. Lewisi*, but the flagellum appears sometimes to divide in its whole length. During division the nucleus elongates, and the chromatin collects at its two extremities. The parasites remain mobile during division, the mobility being somewhat diminished for the time. The parasites resulting from the division are of about equal size.

*Agglomeration.*—Laveran and Mesnil observed agglomeration in *T. Brucei* similar to that described above in *T. Lewisi* (see Fig. 78). When agglomeration occurs in pairs the appearance simulates conjugation (see Fig. 80). The agglomerated parasites may detach themselves or remain permanently associated until degeneration sets in.

*Involution Forms.*—When blood containing *T. Brucei* is placed on ice or in other unfavourable conditions the parasites contract and become rounded; thus altered they

may become agglomerated, and so give rise to masses which simulate plasmodia. Parasites that have become rounded are not necessarily dead, but when injected into a susceptible animal may recover and set up infection. In such cases the period of incubation is lengthened.

If the unfavourable conditions are continued the parasites die, and no longer react to stains in the same way as

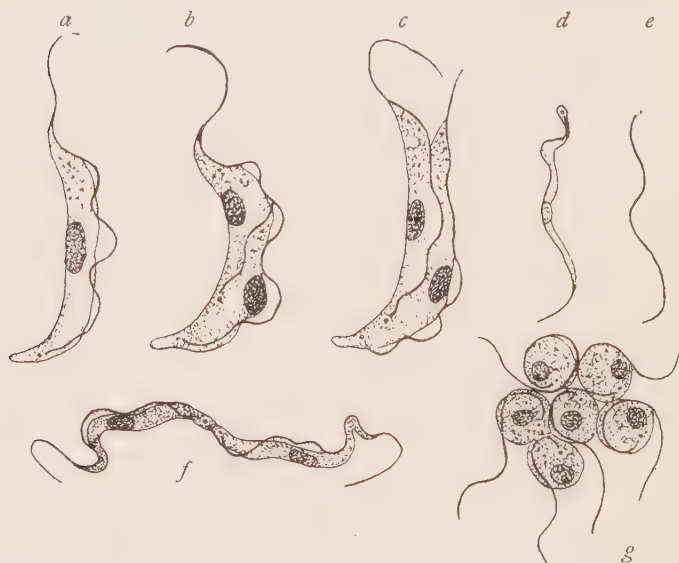


FIG. 80.—*TRYPANOSOMA BRUCEI*. (After Laveran and Mesnil.)

*a*, Full-grown parasite ; *b*, *c*, stages of division ; *d*, *e*, involution forms ; *f*, agglomeration simulating transverse division ; *g*, agglomeration simulating plasmodium formation.

healthy ones. The protoplasm disappears, leaving at last only the flagellum and the 'centrosome.'

*Differential Points.*—In rats Koch found that it was easy to set up a mixed infection of *T. Lewisi* and *T. Brucei*. This was confirmed by Laveran and Mesnil. The latter authors observe that in the fresh blood of such rats it is not easy to distinguish the parasites of the two kinds,

except in the multiplying forms, and the latter are often absent in the *T. Lewisi*. In well-stained preparations the distinction is not difficult.

*Disputed Points.*—The nature of the corpuscle in which the flagellum terminates after forming the margin of the undulating membrane is not yet settled. Wasielewski and Senn\* observe: 'Our preparations show clearly that it is an organ belonging to the periplast in close relationship with the undulating membrane. The thickened edge of the latter can always be traced to this body, and is to all appearance the starting-point of the movements of the membrane and the flagellum.' The authors compare it with the dark-staining body described by Grassi and Schewiakoff at the base of the second and third pair of flagella in another flagellate, 'Megastoma.' Strasburger has described similar bodies in the swarm-spores and gametes of some filamentous algæ (*oedogonium*, etc.), and in these cells he does not regard them as centrosomes, because they appear to take no part in nuclear division.

The interpretation of the radicular corpuscles of *trypanosoma* as centrosomes has, however, in its support the fact that in the ciliated cells of metazoa the 'basal bodies' are both centrosomes and the starting-points of the cilia. Laveran has adopted the view that they are centrosomes.

Plimmer and Bradford† term the body provisionally a micronucleus. This latter name has so definite a meaning for the ciliata (*q.v.*) that it is somewhat hazardous to apply it to the structure under consideration.

*Modes of Multiplication.*—The only established mode of reproduction is that by longitudinal division. In a preliminary paper Plimmer and Bradford‡ described transverse division also, but they have since recognised that this was an error. In their later paper they describe conjugation and amœboid and plasmodial forms. Laveran and Mesnil refer the former to the agglutination described above, and the latter to processes of involution. It is clear that these points require further investigation.

*Mal de Caderas.*§—A parasite closely resembling the *Trypanosoma Brucei* has been found to cause a disease

\* *Zeitschrift für Hygiene und Infektionskrankheiten*, May, 1900: 'Flagellaten des Rattenblutes.'

† *Quart. Journ. Microsc. Sci.*, March, 1902.

‡ *Proc. Roy. Soc.*, vol. lxx.

§ O. Voges, Buenos Ayres, *Zeitschr. für Hygiene*, 1902, Bd. 39, Heft 3.

in cattle in South America; the name is derived from the chief symptom being paralysis of the hinder extremities.

*Trypanosoma Theileri*.—Described by Lieutenant-Colonel Bruce from specimens sent from the Transvaal by Dr. A. Theiler. They were obtained from cattle, and are almost twice as large as *T. Lewisi* and *T. Brucei*. They are harmless to horses, dogs, goats, etc.; in calves they produce fever (*Proc. Roy. Soc.*, February 27, 1902).

*Trypanosoma in Man*.—Nepveu in Algeria, Forde and Dutton\* in Gambia, have found trypanosomata in man's blood. The latter has given a clear account of his case. The patient was an Englishman, aged forty-two, stationed in Gambia, West Africa. The chief clinical features of the case as recorded by Dutton were :

- ‘ 1. Its chronic course.
2. The general wasting and weakness.
3. The irregular rises of temperature, never very high, and of a relapsing type.
4. The local œdemas.
5. The congested areas on the skin.
6. The enlargement of the spleen.
7. Constant increased frequency of pulse and respiration (hurried breathing).

‘ This condition still persists; the general weakness has increased, in consequence of which the patient has been again invalided home, and arrived in England a few days ago.

\* J. Everett Dutton, *Brit. Med. Journ.*, September 20, 1902. In Dr. Dutton's absence, Dr. H. E. Arnett kindly gave me permission to use Dr. Dutton's illustration.

*'The Parasite observed in the Blood* (Fig. 81).—Although many slides were made, and fresh preparations of the blood examined throughout the time the patient was under observation, no malaria parasites were discovered. The first examination of the blood was made on December 15. On that day I made three fresh pre-

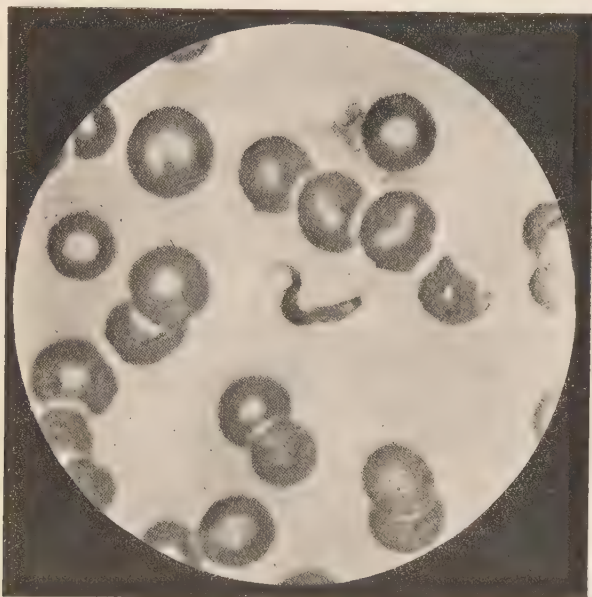


FIG. 81.—TRYPANOSOMA IN HUMAN BLOOD. (Dr. J. E. Dutton's preparation.)

parations, using a  $\frac{3}{4}$ -inch square cover-glass. In these preparations I observed altogether three parasites, presenting all the characteristics of the trypanosomata.

*'In fresh blood the parasite appears as a very minute wormlike organism, very difficult to see with a magnification of 300 diameters, especially in this case, when only a few are present in a preparation, and the parasite is entangled*

amongst a clump of red corpuscles; it glides along fairly rapidly among the red cells, imparting very little movement to them. When the movements have slowed down, one end of the organism is seen to be drawn out into a whiplike process—the flagellum; the other end is bluntly conical; attached along one side of the body is a transparent flangelike process—the undulating membrane; the body itself is short and thick, and its substance granular. There is a highly refractile spot situated near the posterior end (vacuole).

‘ The parasite usually is seen progressing with the flagellum, which represents the anterior end, in front, but at times, when an obstruction is insurmountable, it shoots backwards for a short distance, with the blunted end (posterior) forward. Progression is brought about by wavelike motions started in the flagellum and communicated along the undulating membrane, also by contractions of the body protoplasm. The parasite in rapid motion moves in a screwlike manner, its body rotating around the longitudinal axis, so that the undulating membrane appears as if it were spirally arranged around the organism.

‘ On one occasion I observed the process of phagocytosis take place on a slide one hour after the blood was drawn; a mononuclear leucocyte had partially englobed the trypanosome, only the flagellum and a small portion of the anterior part of the body remaining free.

‘ In fresh preparations, ringed with vaseline, the parasites appear to die in a few hours after the blood is drawn (one observation three hours). In such preparations, left overnight, I was never able to find the trypanosoma again in the morning. Atmospheric temperature varied from 90° F.

in the day to 65° F. during the night. I was unable to obtain an exact measurement of the parasite in the fresh state. . . .

‘The length of the parasite, in stained preparations, including the flagellum, varied from 18  $\mu$  to 25  $\mu$ .’

For further details the original article should be consulted.

Patrick Manson\* has related another case, that of a lady aged forty, who every tenth day had fever which lasted for three days, erythema in spots and patches, disturbance of vision, pains in the joints, etc. The parasites were found after the patient had returned to the Congo. Both the patient and her husband attributed the illness to a bite, presumably of an insect, on the patient’s foot. Manson suggests that the bite was possibly that of a tick (*Ornithodoros Moubata*), the bite of which is known to be followed by fever, abdominal pain, and purging.

\* Manson, ‘Trypanosomiasis on the Congo,’ *Brit. Med. Journ.*, March 28, 1903.

## CHAPTER VIII

### CILIATA

THE ciliata are named from the possession of motor appendages in the form of vibratile cilia. Essentially a cilium is akin to the flagellum of the flagellates, but it differs in having a simpler to-and-fro movement and in its smaller size; whilst a flagellate has never more than six to eight flagella, the body of a ciliate may be covered with countless cilia. In a subclass of the ciliata, the *suctoria*, the cilia are present only in the early stages of the animal's life, the adult animal being provided with hollow tentacles.

In general form the ciliata are extremely varied, but the ovoid appears to be that from which all are derived. The outer layer of the animal, or cell-membrane, constitutes a definite cuticle. It is pierced by a mouth, and usually by an anus. The ectoplasm forms a thin but firm layer, and, together with the cell-membrane, constitutes a firm cortex. The endoplasm occupies the greater part of the animal's body, its outer layer (cortical plasma) being firmer than the more abundant central plasm. The body of the animal is capable of many changes of form, but when at rest the shape characteristic for any given species is returned to. Taking, then, the ovoid as an average type, it is found that during progression the same

extremity is always directed forward. This end is termed 'superior.' In free kinds the animal sometimes creeps along the surface of objects about it, and in doing so one surface becomes flattened. This is termed its ventral surface. On the upper aspect of the ventral surface, and not far from its left border, is a longitudinal depression, the *peristome*, the left border of which is steep and oblique, the right sloping. At the lower end of the peristome is the mouth, which opens into a passage (the pharynx), which leads into the interior of the endoplasm, being directed towards the central part of the animal.

The cilia are disposed in regular longitudinal lines: they are prolongations of the ectoplasm through the cell-membrane. At the left side of the peristome the adjacent cilia are fused together into triangular lamellæ—the *membranelles*—motor organs that by their constant movement keep up a flow of water and nutrient particles towards the mouth. Neither the peristome nor the membranelles are constant in the ciliata.

The endoplasm is constituted by a network of hyaloplasm, in the meshes of which is the more liquid paraplasm. The latter contains the usual granules, or microsomes.

Immediately beneath the ectoplasm is the contractile vacuole, which opens by a pore on the external surface. In some species there are two contractile vacuoles.

*Food Vacuoles.*—The larger particles of food are contained in vacuoles, whilst the products of assimilation assume the forms of grains of 'zooamylum.' Excretory granules, and possibly fat-drops, also occur in the endoplasm.

The *nuclear body* consists of two structures, the macronucleus and the micronucleus (Fig. 82, N, *n*).

The *macronucleus* is voluminous, rounded, or elongated in form, and it resembles an ordinary cell-nucleus in appearance, though it differs from this in not having either a nucleolus or a centrosome. The nuclear membrane and indications of a faintly-staining chromatin network are present; the nuclear juice also becomes stained.

The *micronucleus* is a minute (at most 3 to 4  $\mu$ ) body,

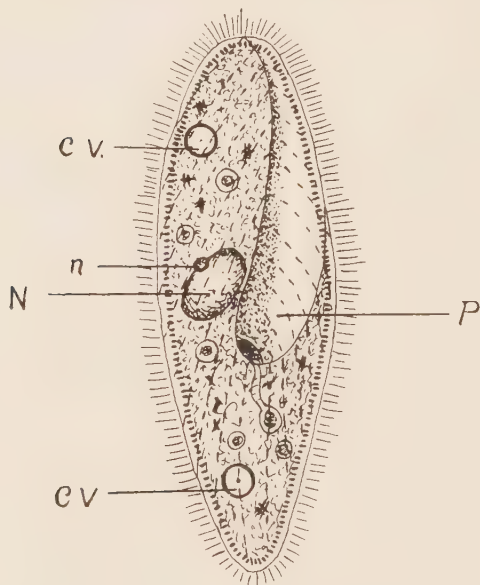


FIG. 82 (*bis*).—A PARAMÆCIUM.

N, macronucleus; n, micronucleus.

situated close to the macronucleus. It possesses a membrane, and on close inspection shows a finely granular structure. The micronucleus was at first termed a 'nucleolus,' but the body appears not to have the physiological significance of a nucleolus. The fundamental form of both the macronucleus and the micronucleus

is a spheroid, but elongated and complicated forms occur.

*Reproduction* occurs solely by division, which may occur (a) in the free state, or (b) within a cyst, or (c) it may follow conjugation.

In simple division the macronucleus first assumes a rounded form, then elongates and becomes constricted, and finally separates in the middle, the nuclear membrane remaining intact. The micronucleus divides by a simple mitosis without centrosomes. As the two sets of chromosomes separate, the part of the membrane of the micronucleus which connects the two new micronuclei becomes greatly drawn out, and is called the 'gubernaculum.' The new micronuclei then become detached from the gubernaculum, and the latter disappears.

*Division of the Protoplasm.*—Whilst the process of division is taking place in the two parts of the nucleus a new mouth and its annexes (peristome, etc.) appear in part of the animal's body, and a constriction forms between the part bearing the old and the part bearing the new mouth. This constriction deepening, the separation of the two daughter-cells, is completed. In favourable conditions two or three successive divisions may occur in twenty-four hours.

*Division within a Cyst.*—Before division the animal loses its cilia, and becomes rounded. Division into two or four then follows, the nuclear processes being the same as in simple division in the free state.

*Senile Degeneration.*—When repeated divisions (150 to 200) have occurred, an exhaustion of vitality occurs in the later generations, as evidenced by the individuals not reaching their normal size, and later still (after 300

generations) by the micronucleus being imperfect or absent. In the latter case the animal is incapable of reproduction.

*Conjugation* occurs in colonies that have undergone the senile degeneration described above. The animals adhere and fuse together in pairs, mouth to mouth (Fig. 83, *d*). When the micronucleus has not disappeared or undergone marked degeneration striking changes occur. Both the macronucleus and the micronucleus undergo subdivision. All the fragments of the macronucleus degenerate and disappear, and all but one of those of the micronucleus have the same fate; the remaining one of the segments of the original micronucleus divides into two parts, which have an important destination: one crosses over from each animal to the other, and fuses with the remaining micronuclear segment in the apposed animal, forming a single nuclear body in each of the conjugating animals, which then separate, and the nuclear body in each subdivides into a new macronucleus and a new micronucleus. After a short period of rest the mouth and its appendages are reformed, and the rejuvenated animal is then capable of starting a new series of agamic individuals.

*Merotomy*.—As in other protozoa, it has been found that if a ciliate has been cut into two parts the one containing the nuclear body is capable of regenerating all the parts that have been removed, and reforming a complete animal, whilst the non-nucleated segment soon perishes.

Many ciliata live in that modified state of parasitism to which Van Beneden applied the term *Commensalism*. Such creatures live within the bodies of larger animals, like parasites, and they often, like true parasites, possess a

modified organization. They are, however, not true parasites, because, instead of feeding on the tissues or juices of their host, they share its food or live upon the refuse of its body. Such creatures may harm their host by encumbering important organs—*e.g.*, the gills of fishes. In other cases they appear to cause no harm, as appears to be the case with the ciliata of the ruminant's stomach.

### *Classification.\**

The subclass ciliata is subdivided in accordance with the arrangement of their ciliæ.

ORDER 1. *Holotrichida*.—Ciliates in which the cilia are similar, and distributed all over the body, with, however, a tendency to lengthen in the neighbourhood of the mouth. Trichocysts are always present, whether distributed about the body or limited to a region—*e.g.*, *paramæcium*.

The trichocysts are shown in Fig. 82 as vertical rods in the deeper part of the cortical layer. They are protruded and separated from the body of the animal when it is irritated—*e.g.*, by an electric current.

One of the most strikingly modified families of this order are the Opalinidæ—*e.g.*, *Opalina ranarum*—which occurs in the large intestine of frogs, and which has a great number of nuclei, that are neither macro- nor micro-nuclei.

ORDER 2. *Heterotrichida*.—Characterized by the possession of a uniform covering of cilia and an 'adoral zone,' consisting of short cilia fused together into mem-

\* For purposes of classification the terms 'infusoria' or 'ciliophora' are sometimes used to include the ciliata proper and the suctoria, which are subdivisions of the class.

branelles. The *Balantidium coli*, considered below, is an example of this order.

ORDER 3. *Hypotrichida*.—The cilia are limited to the ventral surface, and the animal is flattened dorso-ventrally. The cilia are frequently fused together to form larger appendages, the cirrhi, and there is an adoral zone of membranelles.

ORDER 4. *Peritrichida*.—Usually of cylindrical form or cup-shaped, and the cilia are limited, as a rule, to the adoral zone, but secondary rings of cilia may be present. To this order the common *vorticella* belongs.

### *Balantidium Coli.*

This parasite (Figs. 83-85), discovered by Leeuwenhoek, was described more fully by Malmsten\* in the stools of a man who had for two years subsequent to an attack of 'cholera' suffered from constipation, alternating with diarrhœa.

Leuckart found that *B. coli* occurred constantly also in swine, which appear to be its normal hosts, without being in any way inconvenienced by it. Man, on the contrary, appears only occasionally to be infested, when abnormal conditions of the intestine favour its life. The body of the animal is oval; the short peristome is at the blunt (anterior) end: it is funnel-shaped, and ends in a short pharynx. The animal measures from 70 to 100  $\mu$  by 50 to 70  $\mu$  in the fresh state. After fixation it is smaller. The N. is bean-shaped, the n. lies close to it. Two contractile vacuoles are present. The plasma contains globules of mucus, fat, and sometimes blood-corpuscles.

The parasites are of somewhat larger dimensions in the

\* Malmsten, *Virchow's Archiv*, 1857.

pig than in man, so it is not yet definitely ascertained whether they are the same species or not. The most recent work on the subject of *B. coli* is that of Klimenko.\*

The patient was a labourer, who had suffered from diarrhoea for three or four years. Blood was often present in the stools, and balantidia were always found. In spite



FIG. 83.—BALANTIDIUM COLI.

*a*, Nucleus divided ; *b*, commencing ; *c*, advanced segmentation of parasite ; *d*, conjugation. (From Leuckart's 'Parasites of Man,' Hoyle's translation.)

of large injections of quinine solution, the wasting and weakness of the patient increased; the face and limbs became œdematous, and the patient died. After death the mucous membrane of the stomach and small intestine was found to be red and injected, but no balantidia were

\* Klimenko, Ziegler's *Beiträge*, Bd. xxxiii., Heft 1 and 2, 1903.

found on microscopic examination. In the large intestine there were ulcers—one in the ascending, two in the transverse colon, and three in the rectum. The micro-



FIG. 84.—BALANTIDIUM COLI. (After Klimenko.)

The parasite as seen when stained.

scope showed balantidia, surrounded by inflammatory infiltration (Fig. 85), in parts of the mucosa distant from the ulcers, whilst in the ulcers themselves only a few

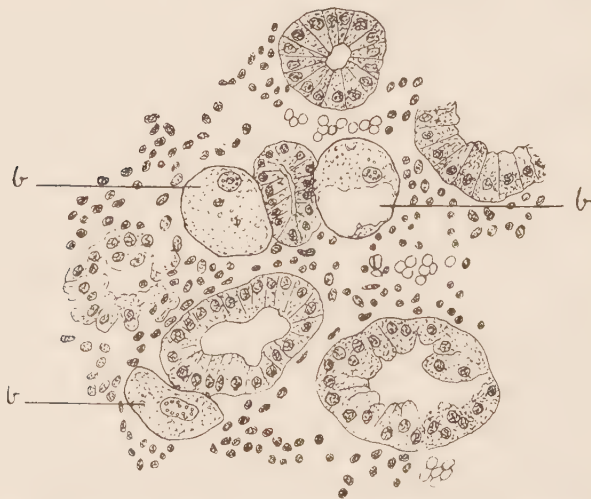


FIG. 85.—A SECTION OF THE MUCOUS MEMBRANE OF THE LARGE INTESTINE CONTAINING THREE BALANTIDIA, *b*, *b*, *b*. (After Klimenko.)

degenerated parasites were found. Klimenko is inclined to regard the parasites as the cause of the disease.

Russell and Buzzard\* have found living balantidia in

\* A. E. Russell and G. F. Buzzard, *Path. Soc. Trans.*, 1899.

a small cyst in the liver of a man who died of cancer of the stomach.

*Suctoria, or Tentaculifera.*

The suctoria are a relatively small group of the protozoa. They begin life as ciliates of the holotrichous type. As they reach maturity they lose their cilia, and develop stiff tentacles, which are traversed by a canal, which is continued into the endoplasm.

The walls of the tentacles are formed by a prolongation of the ectoplasm of the animal. Some of the tentacles terminate in a knob; others are devoid of this terminal enlargement.

Many of the suctoria live by preying on other ciliates. When a suitable organism touches one of the tentacles the latter adheres to it, and the neighbouring tentacles bend towards the prey, and, becoming adherent, hold it fast. The tentacles appear to secrete a poison: for when a ciliate is thus captured the movement of its cilia ceases. The victim thus secured, the tentacles pierce its ectoplasm, and then the endoplasm is sucked through their channels. When the more fluid parts have been absorbed, the remains are released by the tentacles slowly detaching themselves.\*

The tentacles of the suctoria have been compared to the mouth of other ciliata, multiplied and placed at the end of a tentacle. During encystment the tentacles are retracted.

*Internal Structure.*—The chief internal parts—macro-

\* Délage and Hérouard recall the observation of Clarapède and Lachmann that one of the ciliata (stylonychia), when captured by an acineta, divided into two parts, one of which escaped, a mode of defensive autotomy worth recording.

and micro-nucleus—are similar to those of other ciliates, and they behave similarly during conjugation. The contractile vacuole is usually a conspicuous feature in the living suctorian (Fig. 86).

*External Form.*—The majority of the suctoria are fixed by a peduncle, which is not like that of a vorticella, a prolongation of the animal's body, but a chitinous secretion

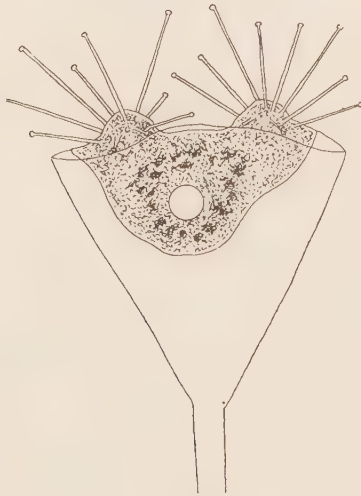


FIG. 86.—A COMMON PEDUNCULATED SUCTORIAN, ACINETA. The animal rests in a stalked theca. A contractile vacuole and pigment masses are present.

similar to that which forms receptacles in other species of ciliates. The peduncle is usually dilated at one end to form a receptacle for the animal's body, and at the other, where it is attached to some submerged object, it is slightly expanded.

Certain species are able to detach themselves from their peduncle and lead a free life. Others of the suctoria are devoid of any trace of a peduncle. Some of these, such as

*Sphærophrya magna* (Fig. 5, p. 8), are of relatively considerable dimensions, and are able to prey actively on other ciliates. Particular interest attaches to the smaller forms of sphærophrya, which live as endo-parasites in various ciliates. Thus the young *S. pusilla* is a free-swimming tentaculate suctorian.

In this condition, if it meets with a paramæcium, it adheres to the surface, loses its cilia, and gradually passes into the interior of its host, leaving a small channel (*geburtsöffnung*) open to the exterior. In the interior of the paramæcium the tentacles are withdrawn, and successive divisions take place, and in this way as many as fifty young parasites may be formed. The earlier divisions are of the ordinary binary type; later, budding occurs, resulting in minute swarm-spores, provided with both cilia and tentacles. In this form the brood escapes, and is ready to infect a fresh host (Fig. 6, p. 9).

It is to those of the suctoria which live as intracellular parasites that most attention must be given by pathologists. In certain sarcomata intranuclear bodies that closely resemble suctoria in form occur.\* Bodies that have been described in certain protozoa as peculiar nuclear structures have later been proved to be suctorian parasites. With a view of forming a basis of comparison of suctorian forms with cell-forms met with in morbid growths, it is important to consider the nuclear forms met with in suctoria, especially in their reproductive phases.

*Reproduction.*—Transverse division into two equal or subequal parts occurs, as in the ciliata in general. The tentacles are retracted before division occurs. In peduncu-

\* *Centralblatt für Bakt.*, abt. i., bd. xvii. (1895), plate vi., fig. 5.

lated forms one segment remains attached to the peduncle, and has only to put out fresh tentacles, but the other segment needs to find a suitable place for becoming attached, and for this purpose the whole surface becomes covered with cilia. As soon as a suitable spot is touched the animal becomes attached at part of its surface, pushes out tentacles, and loses its cilia.

In such of the suctoria as have both macro- and micro-nucleus the two divide separately. The macronucleus assumes an elongated biscuit-shape, the two dilated ends



FIG. 87. — MULTIPLE BUDDING IN A SUCTORIAN, EPHELOTA GEMMIPARA. (After R. Hertwig, from Lang.)

Branches of the nucleus of the parent pass into the buds.

being joined by a long and very narrow neck. The chromatin, after passing through a spirema stage, collects at the two extremities, and the narrow neck uniting the two extremities separates at its middle. The micronucleus divides, by mitosis, but without centrosomes. The membrane is drawn out as a narrow link (gubernaculum) between the two daughter micronuclei, and, becoming detached from them, is absorbed. Conjugation occurs as in the ciliata. It has recently been fully described in *dendrocometes* by Hickson and Wadsworth.\* In one of

\* Hickson and Wadsworth, *Quart. Journ. Microsc. Anat.*, February, 1902.

the suctoria, *dendrosoma*, though it remains unicellular, the body of the animal is much branched, and the macro-nucleus follows these ramifications.

Multiplication by unequal segmentation in the form of budding occurs in two variations, exogenous and endogenous.

In *exogenous budding* (Fig. 87) a number of papillæ arise

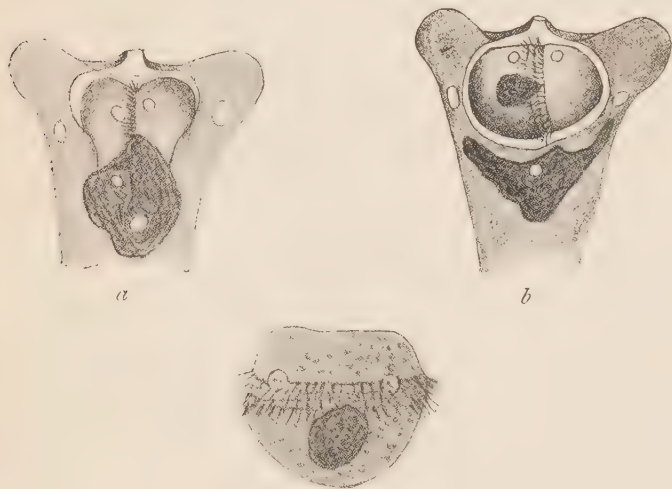


FIG. 88.—STAGES OF BUDDING IN A SUCTORIAN, TOKOPHYRA QUADRIPARTITA. (After Bütschli, from Lang.)

*a*, Nuclear division with partial separation of embryo; *b*, complete separation of embryo; *c*, free swimming embryo after its escape from the cavity in the body of the parent.

on the outer surface, and each receives a part of the dividing nucleus, and becomes detached as a ciliated embryo, as in the simple binary division just described.

*Endogenous budding* (Fig. 88) arises by the bud becoming invaginated into the body of the parent cell before it is detached. The embryo develops a circle of cilia, and becomes

free either by dilating the orifice of the cavity of the invagination, or by the invaginated part becoming pushed back again, the bud being thus converted into the exogenous type before separation. When the endogenous buds are multiple, each may be lodged in a separate recess, or they may all arise from the walls of a single large depression. In the latter case a close resemblance to swarm-sporing may result.

## CHAPTER IX

### THE DISEASES OF THE PROTOZOA

It is not without interest to inquire what diseases the protozoa themselves are subject to. In his classical work on inflammation, Metchnikoff\* has recorded some observations of great interest on this subject. In the interior of an amœba certain round cells to which the author has given the name *microsphæra*, instead of being digested like the diatoms, which the amœba had also engulfed, multiplied by successive divisions in the protoplasm of their host, which, having ejected the undigested remains of the diatoms, became weaker and finally perished. The *microsphæra* had the power of resisting the digestive action of the amœba; in other words, it was pathogenic as regards its host.

Among the infusoria several species have long been known occasionally to contain rod-shaped bodies, which their discoverer, J. Müller, mistook for spermatozoa. They were subsequently looked upon as parasitic bacteria. Metchnikoff and Hafkine† found that they belonged to a special group of vegetable organisms, distinct from the bacteria and allied to the yeasts. The

\* Elie Metchnikoff, 'Leçons sur la Pathologie Comparée de l'Inflammation,' 1892.

† *Annales de l'Institut Pasteur*, 1890, p. 148.

term 'monospora' has been applied to them. Similar parasites invade one of the lower crustaceans, *Daphnia*, and cause its death. Among the protozoa the same parasites have been found to infect certain paramæcia, in which sometimes the macro-, sometimes the micronucleus is the seat of the parasitic invasion (see Fig. 89). In spite of the invasion of such vital parts, some of the infected paramæcia are capable of division, though many

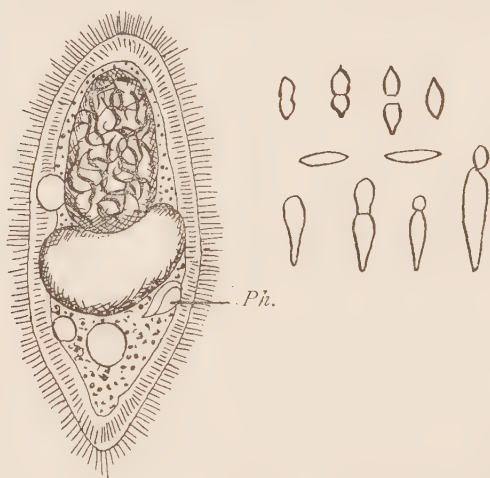


FIG. 89.—A DISEASED PARAMÆCIUM. (After W. W. Hafkine.)

The micronucleus is greatly hypertrophied and filled with the spirillum-like monospora undulata; to the right is a simpler form of the same kind of parasite; *Ph.*, pharynx of the host-cell pushed aside.

die of the disease. When an infected paramæcium divides, a certain number of the parasites escape into the protoplasm of the host, and are ejected therefrom. The parasites undergo transverse division which is sometimes unequal (budding; see Fig. 89). These earlier stages are observed by crushing a recently infected animal under the microscope. In a later stage the monosporæ become

converted each into a spore. This change is characterized by their becoming more highly refracting and ceasing to take the ordinary stains. Hafkine was unable to infect paramæcia artificially; the monosporæ were incepted, but rejected by the protoplasm. It would appear that it is necessary for these parasites to reach and enter the nucleus, and thus escape the digestive action of the protoplasm, in order to thrive. Another kind of parasitic fungi that infect certain protozoa are the synchytriaceæ. With these have been confused certain parasitic protozoa, as already explained (p. 25). Certain synchytrians\* enter the body of *Euglena viridis*, a flagellate (Fig. 3, p. 5), and after causing a diminution of the chlorophyll of the host and a deposit of pigment, break up into zoospores and cause the death of the euglena.

Certain of the suctoria have already been referred to (p. 9, Fig. 6) as parasitic in other protozoa of their kin. Metchnikoff explains the power of these intracellular parasites to resist the digestive power of their hosts by supposing that the parasites secrete toxic substances that paralyse the digestive and also the expulsive functions of their hosts.

These instances of parasites that affect the protozoa themselves are of interest in that they throw light on the phenomena of parasitism in general, and on the properties of the protozoa themselves. As knowledge increases it may prove that some of the protozoa have certain diseases in common with higher animals, and, indeed, that protozoa may serve as intermediaries in communicating to man some of their own diseases.

\* Metchnikoff, *loc. supra cit.*, p. 28.

## CHAPTER X

### METHODS

IT will, perhaps, be found more convenient to collect together here the more serviceable of the methods of investigation of the protozoa in a separate chapter than it would have been to have given them in connection with the particular parasites to which they have been applied. In this way the description of methods will serve as a basis for reviewing the descriptive matter that has preceded it in the book. A general knowledge of histological and bacteriological methods will be presupposed. Methods are, of course, entirely subservient in the pursuit of knowledge, and, though the value of sound methods is of the greatest importance, the first requisite is to understand the nature and mode of life of the organisms that are under investigation or are being sought for. This chapter does not pretend to be in any way exhaustive, but only to give examples of methods that have hitherto proved most useful.

The examination of the living organisms holds the foremost place in the investigation of the protozoa. This is usually best carried out in the fluids in which they occur naturally—*e.g.*, *Amæba coli* in the intestinal liquid, malarial parasites and other blood-parasites in blood or blood-serum, etc.

Physiological salt solution (0.75 per cent. NaCl in distilled water) may be used either alone or to dilute the natural fluid. Where the protozoa are of relatively large size, the cover-glass should be provided with points of wax or paraffin, in order to prevent pressure on the organisms; and if some degree of pressure is required to prevent them from moving out of the microscopic field, the height of the wax supports may be reduced by pressure with a warmed glass rod on the upper surface of the cover-glass.

When the parasites of warm-blooded animals are under investigation, it may be necessary to examine them at the temperature at which they exist naturally. For this purpose a warm stage (preferably Stricker's) is required. On the other hand, the life of the flagellates of tsetse-fly disease is prolonged by keeping the blood in which they are contained in a refrigerator.

Living protozoa are best examined in diffuse daylight, directed through the microscope by a plane mirror; a diaphragm, but no condenser, being used.

It is frequently necessary to watch from day to day the changes that take place in protozoa when kept in a moist chamber. The most convenient way of doing this I have found to be effected by placing a little of the material containing the protozoa in the hollow of the slides used for hanging-drop preparations. A drop of water or nutrient liquid may be added. The slides and the liquid should be previously sterilized, and when the material to be investigated has been placed in the hollow of the slide, the preparation should be transferred to a sterilized Petri's dish containing a few layers of moist, sterilized filter-paper.

*Permanent Preparations.*—A good fixation is necessary, and of all fixing fluids\* the most generally useful is solution of perchloride of mercury, made by saturation of boiling 0·75 salt solution, the excess of the reagent being allowed to crystallize out: this fluid should always be kept in stock. At the time of using a few drops of glacial acetic acid are added. This reagent gives equally good results with hæmatoxylin and with many aniline stains—*e.g.*, gentian violet and Ehrlich-Biondi. When used to harden tissues, the fragments should not be more than 2 millimetres in thickness, owing to the penetration of the reagent being limited. As an example of the use of perchloride solution when applied to cover-glass preparations, the details given by Siedlecki† of his investigation of the coccidia of the cuttle-fish may be quoted. I have found that it answers well for the investigation of gregarines and other large protozoa, as well as smaller ones. In fixing gregarines to the cover-glass it is of advantage to add a few drops of filtered egg-albumin and glycerin mixture when they are suspended in physiological salt solution.

The angles of a No. 1 square cover-glass are fused in a Bunsen flame to make four tiny knobs, that prevent flattening of the parasites. If the latter are contained in epithelium, the tissue is teased out with a little filtered sea-water‡ or intestinal juice, and spread in a thin layer on the cover-glass, prepared as described above. The preparation must not be allowed to become dry, but is to

\* For information concerning histological fluids and processes, 'The Microtometist's Vade-mecum,' by A. B. Lee, should be consulted.

† Siedlecki, *Ann. de l'Inst. Pasteur*, 1898, p. 799.

‡ Substitute physiological salt solution where the host is not of marine life.

be immediately inverted, and floated on the fixing fluid contained in a watch-glass. There must not be too much liquid in the preparation, or some of it falls away when it is floated on the solution. Siedlecki used a saturated solution of perchloride of mercury in sea-water, 3 to 5 drops of glacial acetic acid being added to 100 c.c. of this solution. The preparation is to remain for from half to one hour in the fixing solution. It is then washed for at least four hours in water, that should be changed several times, and subsequently washed for from ten to thirty minutes in each of the following successive alcoholic baths—30, 50, 70, 90 per cent. A few drops of an alcoholic solution of iodine should be added to the alcohol of the 70 or the 90 per cent. bath.

For staining Siedlecki used Böhmer's\* hæmatoxylin, in weak solution, in distilled water. The staining was continued for from twelve to twenty-four hours, and differentiated in alcohol containing traces of hydrochloric acid. The colour changes to a red tinge, but it is restored to a bluish violet by washing in 50 per cent. alcohol, slightly ammoniated.

For sections Heidenhain's iron-alum hæmatoxylin gives good results. Ehrlich's acid hæmatoxylin, Delafield's

\* BÖHMER'S HÆMATOXYLIN (ALUM).

1. Hæmatoxylin crystals	...	...	1 gramme.
Absolute alcohol	...	...	10 c.c.
2. Alum	...	...	20 grammes.
Distilled water	...	...	200 c.c.

Cover the solutions, and allow them to stand overnight. The next day mix them, and allow the mixture to stand for *one week* in a wide-mouthed bottle lightly plugged with cotton. Then filter into a bottle provided with a good cork. The solution is then ready for use. It improves by 'ripening.'

hæmatoxylin, Ehrlich-Biondi triple stain, and gentian violet, have all been found to give good results with preparations fixed with perchloride.

It is not necessary to give here well-known histological methods, but the methods that gave such brilliant results in the hands of M. Heidenhain\* after fixation with perchloride solution may be briefly referred to.

*Iron-Hæmatoxylin Method.*—(1) The sections are placed for two hours in a 4 per cent. solution of ferric alum  $(\text{NH}_4)_2\text{Fe}_2(\text{SO}_4)_4$ ; the crystals should be of a clear purple colour, and dissolved in the cold. (2) Rinse well with water and place for half an hour in a  $\frac{1}{2}$  per cent. solution of hæmatoxylin, made by heating in distilled water. (3) Rinse again with water, and put again in the iron solution till the colour fades; the progress of the decolourisation should be watched under the microscope, a section being placed in tap-water from time to time for this purpose. (4) After decolourisation the sections should be transferred for from three to six hours to running tap-water; this completes the staining, and the preparation can be then mounted in the usual way. By this method nuclei are well stained, and if the treatment with iron and stain is prolonged (twelve to eighteen hours), bacteria in the tissues are stained black. If a counterstain is required, M. Heidenhain recommends the following: Saturated aqueous solution of acid fuchsin 3 parts, saturated aqueous solution of orange G., 150 parts; for use this is diluted with 2 to 4 parts of water.

*Biondi-Ehrlich Heidenhain Stain.*—This is most readily prepared from Grübler's powdered mixture of orange G.,

\* M. Heidenhain, 'Ueb. Kern und Protoplasma,' Kölliker's *Festschrift*, 1892.

fuchsin G., and methyl green, corresponding to Ehrlich's original formula. A 4 per cent. solution of this in distilled water is made. To this 7 c.c. of  $\frac{1}{2}$  per cent. solution of acid fuchsin may be added at the time of using. The sections, before being stained, are treated with 1 per cent. acetic acid, then tincture of iodine, for ten to fifteen minutes, and then rinsed with alcohol.

*Special Methods.*—Observations on the cultivation of amœbæ have been made earlier in this work (p. 38), but, as a practical exercise, the observations of Tsujitani\* are well worthy of being repeated. Infusions of straw and hay, if examined on the third day, yield, among other organisms, amœboid bodies, which have been named the *straw-amœba*, though they more probably belong to the mycetozoa. Under the microscope they are easily found, side by side with encysted forms. Klemensiewicz† has recently used them as a basis for a study of nuclear division. If an alkaline agar jelly is made with a vegetable infusion (Tsujitani used straw 30 grammes, gigartina prolifera 10 grammes, water 1,000 grammes), is sterilized and filtered, and 1 per cent. of normal soda solution, and 1 to 1.5 agar added, and poured into a tube or a Petri's dish, and allowed to solidify in the usual way, and then the condensation-water is inoculated with material containing straw-amœbæ; bacteria and amœbæ multiply in it. Then, if a streak-culture of *Bacillus coli*, *Staphylococcus aureus*, cholera bacilli, or certain other bacteria, is made on ordinary agar jelly, and a platinum loop of the liquid containing amœbæ and bacteria is placed at the lower end of the streak, the amœbæ advance, devouring the

\* Tsujitani, *Cent. für Bakt.*, 1898, p. 656.

† Klemensiewicz, Ziegler's *Beiträge*, Bd. xxxiii., Heft 1 and 2, 1903.

streak-culture, and leaving behind them the bacteria with which they were originally associated. In a Petri's dish, under the microscope, the advancing zone of amœbæ could be seen in motion, whilst encysted amœbæ were left behind. It was found that the amœbæ did not thrive when the bacteria of the streak were killed by heating to 60° to 70° C. Similar observations were made with amœbæ obtained from dust and soil. But with one kind of bacillus, obtained from hay infusion and killed by heating to 60° C. for ten minutes, Tsujitani thinks that he was able to obtain a pure culture of amœbæ, which had been previously fed upon cholera bacilli and transferred, together with the latter, to silk threads, and desiccated over sulphuric acid; in this way the cholera bacilli were killed, but the amœbæ became encysted and remained alive.

*Differential Stain for Amœba Coli.*—Mallory\* found that for paraffin sections of *A. coli* in tissues a differential staining could be obtained in the following way: The tissue is fixed in alcohol, and the sections stained with a saturated aqueous solution of thionin from five to twenty minutes, washed in water, and differentiated in a 2 per cent. aqueous solution of oxalic acid from a half to one minute, washed in water, dehydrated in 95 per cent. alcohol, cleared in oil of bergamot, followed by xylol and xylol balsam. The nuclei of the amœbæ are then brownish-red, other nuclei blue.

The *hæmosporidia* and *hæmoflagellata*, when examined in the fresh state, require careful preparation of cover-glass and slide; both should be washed in ether and absolute alcohol, and polished with a clean, soft linen cloth to remove all trace of grease. The thin layer of blood should

\* Mallory, *Journ. of Experimental Med.*, vol. ii., 1897, p. 529.

be prevented from evaporating by painting a ring of vaseline round the edge of the cover-glass. If the oxygen of the air is allowed to have access to the preparation by omitting the ring of vaseline, and at the same time the blood is slightly diluted by breathing on the slide previous to inverting the cover-glass on it, the formation of microgametes and ripening of the macrogametocytes is more readily observed. The different stages of sporogony are best observed by teasing out gnats that have fed on malarial subjects and examining the blood, etc., thus obtained.

*Permanent Preparations.*—A rapid method of making a large preparation is to spread on a clean slide a thin layer of blood, drawn after the part it is taken from—*e.g.*, the finger—has been cleansed with alcohol.

Most workers agree that the best way of spreading the film is that first recommended by Jancso and Rosenberger.\* Two slides are cleansed and polished; the drop of blood is transferred to the middle of the edge at the end of one slide, and applied to the middle of the surface near the end of a second slide, which rests on a flat surface. The first slide is slightly inclined from the vertical towards the side on which the blood is, and it is pushed in the opposite direction along the surface of the second slide, keeping the same inclination. In this way the blood forms an even film. If a cover-glass preparation is required, the drop of blood may be caught on the edge of a piece of thin tissue-paper, cut square, half an inch wide; the paper carrying the blood is applied to the cover-glass, and lightly drawn across the surface.

The preparation is exposed in the air sufficiently long

\* Jancso and Rosenberger, *Deutsch. Archiv für Klin. Med.*, vol. cvii., p. 449.

to dry the surface, and then it is placed for one to two minutes in absolute alcohol. After this it is stained in boricated methylene-blue solution, and mounted in the usual way.

Formula :\* Pure medicinal methylene blue, 2 per cent. ; borax, 5 per cent. ; distilled water, 93 per cent.

More careful preparations are necessary for minute examination, and some of the many modifications of Romanowsky's† double stain of methylene blue and eosin have given the best results, although it is not to be supposed that the malaria parasites are refractory to other stains. Thus, Schüffner‡ discovered the granular change that occurs in the red corpuscles infected by the tertian parasite (*Plasmodium vivax*) by using Böhmer's hæmatoxylin in the following way :

(1) Dry the preparation in air shaded from light for from six to thirty hours ; (2) place in a solution containing 1 per cent. formalin with 5 per cent. of glycerin five to ten minutes ; (3) wash in tap-water for from a quarter to one minute ; (4) stain with Böhmer's hæmatoxylin from one to ten minutes, according to density of stain ; finally, wash, dry, and mount in the usual way.

Romanowsky's stain owes its properties to the formation of a new red-staining substance when certain kinds of methylene blue are mixed in solution with certain kinds of eosin. G. Maurer§ recommends the following method of preparing Romanowsky's stain :

\* This and other formulæ are given by D. C. Rees in the *Practitioner*, March, 1901.

† Romanowsky, 'Zur Frage der Parasitologie der Malaria,' Petersburg, 1891.

‡ Schüffner, *Deutsche Archiv für Klin. Med.*, vol. lxiv., 1899.

§ Maurer, *Cent. für Bakt.*, August, 1900.

*Solution 1.*

Methylene blue (medicinal, Höchst)	1	per cent.
Sodium carbonate	...	...
Formalin (to prevent growth of	...	...
moulds)	...	...
	1	per cent.

This solution is allowed to ripen for two or three days in the sun or for eight days at room temperature.

*Solution 2.*

Grübler's eosin (wasserlöslich,			
yellow)	...	...	...
			1
			10
			per cent.

These are the concentrated solutions, and they will keep indefinitely in stoppered bottles; for use they are to be diluted with distilled water in the proportion of 1 part of each stain to 25 parts of distilled water. Equal quantities of these stains are mixed together and used at once. For staining, the films are fixed in alcohol and ether or absolute alcohol for from two to five minutes, then washed in water, and allowed to dry. The diluted stains are mixed, and then applied by laying the slide film downwards in a shallow vessel, one end being raised about 1 millimetre—*e.g.*, by a capillary tube being placed under it—and the stain is run in beneath it, where it is retained by capillary attraction.

After staining from five to thirty minutes, the preparation is rinsed and examined in water. If the staining is sufficient the preparation is well washed in distilled water, and allowed to dry in the air without heat, and mounted in turpentine balsam. By this method the protoplasm of the parasites is stained blue, and their nuclei and those of the leucocytes are stained a bright red. The red cor-

puscles are almost unstained, save for the granular appearance in cases of tertian fever, and this is only brought out by deep staining. Many attempts have been made to simplify this troublesome stain. Louis Jenner,\* by collecting the precipitate which forms when the two solutions are mixed, and dissolving it in absolute methylic alcohol, obtained a combined staining and fixing fluid.

Bradford and Plimmer† found that a 0.001 per cent. solution of erythrosin could be used instead of eosin for the staining of the flagellates of tsetse-fly disease. With the latter parasites Laveran and Mesnil‡ obtained excellent results with the following stain :

A few crystals of  $\text{AgNO}_3$  are dissolved in 50 c.c. distilled water, and precipitated by adding 100 c.c. of soda solution and shaking. The filtrate of  $\text{Ag}_2\text{O}$  is well washed and a saturated solution of methylene blue (medicinal puriss, Höchst) added, and allowed to remain in contact fifteen days. This constitutes Borrel's blue. Two other solutions, one of water-soluble eosin (Höchst), 1 per cent., another of tannic acid, 5 per cent., are also required.

Of these three solutions, 1 c.c. of Borrel's blue, 4 c.c. of eosin, and 6 c.c. of distilled water are mixed together in a shallow dish, and the slide placed therein for twenty to thirty minutes. The preparation is then well washed with water, then for ten to fifteen minutes with the tannin solution, again with water, and then with distilled water ; it is then dried. For examination, the specimen is washed in oil of cloves and then in xylol, and mounted in balsam.

\* Louis Jenner, *Lancet*, February 11, 1899.

† Bradford and Plimmer, *Practitioner*, 1899.

‡ Laveran and Mesnil, *Ann. de l'Inst. Pasteur*.

The specimen keeps better dry than when mounted for examination.

Romanowsky's method also serves for the staining of flagellates.

For the observation of living flagellates precautions similar to those mentioned above for malaria parasites are necessary. To prevent the blood from clotting it may be diluted with solution of neutral citrate of potassium, and to slow the movements of the flagella weak gelatine or cherry gum solution may be added, as suggested by Bradford and Plimmer.

*The Myxosporidia* are observed in the living state in the body-fluids of their hosts or in physiological salt solution. The usual fixing reagents and stains are applicable—*e.g.*, hæmatoxylin and eosin, after sublimate fixing; gentian violet or safranin, after Flemming's fluid. Iron hæmatoxylin is specially recommended by Doflein. Some features are better brought out when glycerin is used for mounting.

For demonstrating the thread-cysts, different reagents answer for different species; thus ether, alkalies, strong mineral acids, boiling water, glycerin, mechanical pressure, etc., have all been used.

The *Sarcosporidia* can be studied in the fresh state in muscle-juice. L. Pfeiffer observed the spores in filtered saliva. Serial sections, stained with iron hæmatoxylin, give very good results. Specimens sent to me by L. Pfeiffer in 1893 are as good now as when they were first received.

*Serumsporidia*.—Calkins found that the *Lymphosporidium truttæ* stained well with basic stains. His preparations, stained with thionin, are very clear and well differentiated.

*Ciliata*.—The cilia are best fixed with osmic acid vapour by inverting a slide on which is some of the liquid containing them over a vessel containing 1 per cent. osmic acid solution. After a few minutes the preparation is treated with a drop of a 10 per cent. solution of sodium carbonate, and covered with a cover-glass and examined. Permanent stained preparations are made as for coccidia, amœbæ, etc.

For the suctoria the usual methods are applicable. S. J. Hickson\* has obtained serial sections of *Dendrocometes paradoxus*, which is constantly present on the gills of the common fresh-water shrimp, *Gammarus pulex*, by embedding portions of the gills in paraffin and cutting sections in the usual way.

\* S. J. Hickson, *Quart. Journ. Micros. Sci.*, February, 1902.

## APPENDIX

### A NOTE ON THE MICROGAMETE PHASE OF COCCIDIUM OVIFORME

SIEDLECKI,\* in his article, 'Étude de la Coccidie de la Seiche,' gives the history of the discovery of the microgamete phase of coccidia as follows:

'A microgamete phase has already been described in various coccidia: Podwyssotzki, Clarke, and especially Simond, in *Coccidium oviforme*; Schuberg, in coccidia of the mouse; Labbé, Simond, and Siedlecki, in coccidia of tritons; Simond, in *C. Salamandræ*; Léger and Hagenmüller, in the genera diplospora and Barroussia; Schaudinn and Siedlecki, in *C. Schneideri* of lithobius. The majority of these savants have described the microgametes as filaments, formed in great part of chromatin on the surface of a large spherical parasite. Quite recently Léger and Wasielewski have found that in certain coccidia—Barroussia, echinospora (Léger), *C. oviforme*, and a coccidium of myriapods (Wasielewski)—the microgametes have a special structure; their body, in the shape of a comma or an elongated club, carries two cilia attached to its anterior extremity, which is always the more developed. The microgametes move by virtue of these cilia.'

This historical sketch is of interest in that it relates to one of the most important vital processes that occur in the protozoa. In the sequence given of the observations

\* Siedlecki, *Annales de l'Institut Pasteur*, 1898, p. 799.

of the microgamete stage in *C. oviforme* there is an error. Podwysotszki's\* account was published in 1895, whilst mine was first published in the *Medical Press and Circular* on August 30 and September 27, 1893. Afterwards I demonstrated the phase before the Pathological Society of London, and in 1895 mentioned the matter in an article in the *Quarterly Journal of Microscopical Science*.



FIG. 90 (*bis*).—SECTION OF COCCIDIAL NODULE IN RABBIT'S LIVER, SHOWING THREE PARASITES IN THE MICROGAMETE PHASE—*i.e.*, STUDDED WITH PERIPHERAL RODS OF CHROMATIN.

This point of precedence is not one of great importance in itself, but it serves to show the kind of criticism that at that time was accorded to original scientific work in an influential professional quarter, and hence it must be considered; for the attitude revealed by this criticism has bearings on other more important subjects. I may there-

\* Abtheilung, D., 'Dermatologie und Syphilidologie': Cassel, 1895.

fore recall my first description of *C. oviforme* in the microgamete phase :

‘The whole of its superficial layer is thickly studded with rods placed perpendicularly to the surface and stained deeply with acid hæmatoxylin. Above and to the right of this is a free parasite, which is divided into three, and the arrangement of the bars of chromatin shows, I think, that the subdivision is the result of karyokinetic activity. Above this, again, is another similar body, devoid of a capsule and divided into two’ (see Fig. 90).—*Medical Press*, August 30, 1893.

Again, in the same journal, September 27, 1893 :

‘I have for some months been studying the phase of the *C. oviforme*, which, as mentioned on p. 210, seems to me

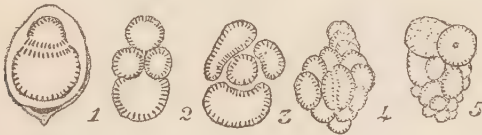


FIG. 91.—COCCIDIUM OVIFORME. STAGES OF THE MICROGAMETE PHASE.

1, Intracellular parasite subdivided into two ; 2-3, free coccidia divided into four ; 4, cell cluster, division still proceeding ; 5, cell cluster resulting from frequent subdivisions. (Zeiss, F.)

to throw important light on the matter. In rabbits' livers which contain numerous large and actively growing lesions, side by side with ordinary forms, are great numbers of parasites in a state of multiplication by direct division. [Some of the stages in this process of subdivision of the *C. oviforme* are shown in Fig. 91.]

‘The coccidia in this modification possess rodlike bars of chromatin, which are placed perpendicularly to the surface, and thus the distinction between nucleus and protoplasm is not present, or, in other words, in this modification the parasites appear to be wholly nuclear in constitution.’

Neither Podwyssotzki nor myself grasped the meaning

of this phase of the parasites; it was Simond who first suggested its sexual character. The account that I submitted to the Pathological Society of London, and the report on the specimens and photographs, may now be quoted from the Society's *Transactions*, 1894, pp. 242, 243.

#### A PHASE OF COCCIDIUM OVIFORME.\*

‘The two sections shown by me to the Society—one on October 17, the other on November 7, 1893—have been photographed for me by Mr. E. W. Roughton and Mr. C. H. Cosens, to whom I would express my hearty thanks.

‘The photograph numbered 1 was taken with a Zeiss's 2 millimetre oil immersion apochromatic and No. 2 projection eye-piece. It shows in the middle of the field a body divided into two unequal segments, the seat of division being marked by two dark lines separated by a light interspace. Passing from the seat of division into the larger segment is a three-sided dark structure, which under a good  $\frac{1}{1\frac{1}{2}}$  oil immersion can be seen to be made up of short rods. Similar short rods are placed all over the periphery of the body. It indicates, I think, a process of further subdivision.

‘The second photograph, taken with a Zeiss's “D” and a longer camera length, shows the same body rather more distinctly, so that with a lens at the seat of division the rods may be made out.

‘The third photograph was taken with a Zeiss's “D” from the section shown to the Society on November 7. One body similar to that shown in the other photograph, only devoid of the process passing from the seat of division into the larger segment, and with a second bud on one side of the larger segment, is present; and also there

\* By J. Jackson Clarke, M.B.

is another large body with two granular buds at one extremity, and elsewhere evidences of further subdivision. These bodies illustrate three of a long series of forms which I have described elsewhere.\* That they are epithelial parasites is shown by their occurrence within some of the epithelial cells. They differ from the ordinary forms of coccidia by the absence of a definitive capsule, by their larger size, and by the presence of definite peripheral rods and other features. The presumption is that they are a form of *C. oviforme*, since in their earlier stages they are indistinguishable from the granular parasites. Photograph 3 shows how strongly they contrast with the granular coccidia, an example of which, well-focussed, is seen near the two bodies already referred to in that photograph. The arrangement of rods at the lines of division leaves hardly any doubt that the process is akin to karyokinesis. I have not been able to distinguish achromatic spindles. Some of Dr. R. Pfeiffer's photographs of the swarm-sporing stage of *C. oviforme* suggest that subdivision of the parasites takes place in some instances before the sickles are formed. I have not been able to find any mention of the definitely grouped rows of rods at the lines of division. After an exhaustive examination I can say that sickles were not present in any of the lesions of the liver I examined in the present case. The small granular bodies were seen free in great numbers in some of the cysts, and they may have the significance of abortive sickles.

'It is more probable, I think, in view of the evident vital activity of the parasites in the phase under consideration, that they are reproductive elements such as I have described in the psorosperms of the ureter and in various cancers. The tissue was fixed in Foa's solution. Section 1 is stained in acid hæmatoxylin; Section 2 the same, followed by eosin.'

\* Jackson Clarke, 'Morbid Growths and Sporozoa,' Figs. 23 and 25

‘November 7, 1903.

‘Report of the Morbid Growths Committee upon Mr. J. Jackson Clarke’s Specimen, showing a “New Stage” in the Development of *C. Oviforme*.’

‘We find in the preparations submitted to us the apparently double forms described by the author. We have not been able, however, to satisfy ourselves that these appearances are not due to the *apposition* of two or more individuals, as distinguished from the *budding* of one, which latter is the interpretation of the author.

‘Viewed laterally, there is, as described, a bright dividing line between the peripheral granules pertaining to the two elements. Individuals of which the apposed surfaces are mutually adapted, and have a similar disposition of peripheral granules, are figured by R. Pfeiffer in his work on the *C. oviforme* of the rabbit (Fig. 18, Plate IX.), and we do not find in the author’s specimens anything not therein represented.

‘(Signed) RICHARD HEBB.

‘SAMUEL G. SHATTOCK.’

No one reading this report would imagine that those who drew it up did not wish it to be understood that R. Pfeiffer had anticipated me in describing this particular phase of *C. oviforme*. How far this is from being the case may be judged from the fact that the bodies seen in the figure referred to in the report are not mentioned by Pfeiffer in the text of his pamphlet or in his description of the figure, but they are accidentally included in a photograph described as: ‘Intestinal coccidia; stained preparation. Magnified 1,000 diameters. Cylindrical epithelium with multiple infection.’ Not a word here about any peripheral rods, and, indeed, the reporters have been obliged to change the term ‘rods’ of my description into ‘granules’ in order to make it possible for them to refer to Pfeiffer’s figure as anticipating my studies. Even had the body in

Pfeiffer's photograph been demonstrably the same as those that I had described, it would not in the least have altered the originality of my account, because Pfeiffer had given no description of it.

To refer to bodies accidentally included in a photograph as being equivalent to a published description is little better than to say that there was nothing novel about the bodies that I described, because they had occurred in the livers of rabbits for ages past. Dr. R. Pfeiffer would, I am sure, be surprised to learn that the first introduction into scientific literature of the microgamete phase of *Coccidium oviforme* had been attributed to him.

We have to thank the committee for the term 'budding'—the formation of microgametes in coccidia may very aptly be termed a process of budding.

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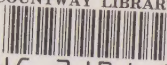








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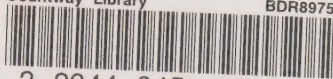
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